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United States Patent *4,022,899*
Adams , et al. **May 10, 1977**

Synergistic local anesthetic compositions

Abstract

A local anesthetic composition comprising a mixture in a pharmaceutically acceptable carrier of a particular toxin, namely, tetrodotoxin or desoxytetrodotoxin, and another compound, generally a conventional local anesthetic compound or a similar compound having nerve-blocking properties.

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References Cited [Referenced By]

U.S. Patent Documents

Other References

chemical Abstracts, vol. 69 (1968), p. 9494w.
Merck Index, 8th Ed. (1968), pp. 126, 174, 246, 312, 531, 875, 882, & 1023.

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Parent Case Text

This application is a division of application Ser. No. 369,302, filed June 12, 1973 (now U.S. Pat. No. 3,966,934), which application Ser. No. 369,302 is a continuation-in-part application of application Ser. No. 206,181, filed Dec. 8, 1971 (now abandoned), which application Ser. No. 206,181 is a continuation-in-part application of application Ser. No. 109,942, filed Jan. 26, 1971 (now abandoned).

Claims

1. An injectable local anesthetic composition having long-lasting local anesthetic effect which is a solution consisting essentially of a pharmaceutically acceptable carrier having dissolved therein

a. an aminoalkyl benzoate local anesthetic compound in a concentration of from 0.05% to 5% by weight of the carrier and

b. a toxin selected from the group consisting of from 0.5 to 10 micrograms of tetrodotoxin per milliliter of the carrier and from 10 to 20 micrograms of desoxytetrodotoxin per milliliter of the carrier.

2. The composition as defined by claim 1 wherein said component (b) is tetrodotoxin.

3. The composition as defined by claim 1 wherein said component (b) is desoxytetrodotoxin.

4. The composition as defined by claim 2 wherein the aminoalkyl benzoate is procaine.

5. The composition as defined by claim 2 wherein the aminoalkyl benzoate is chloroprocaine.

6. The composition as defined by claim 2 wherein the aminoalkyl benzoate is tetracaine.

7. The composition as defined by claim 2 wherein the aminoalkyl benzoate is propoxycaine.

8. The composition as defined by claim 2 wherein the aminoalkyl benzoate is hexylcaine.

9. The composition as defined by claim 2 wherein the aminoalkyl benzoate is cyclomethycaine.

10. The composition as defined by claim 2 wherein the aminoalkyl benzoate is benoxinate.

11. The composition as defined by claim 2 wherein the aminoalkyl benzoate is butacaine.

12. The composition as defined by claim 2 wherein the aminoalkyl benzoate is proparacaine.

13. The composition as defined by claim 1 which further contains an effective amount of a vaso-constrictor.

14. A method of inducing anesthesia in mammals comprising administering to the mammal to be anesthetized an effective amount of an injectable local anesthetic composition having long-lasting local anesthetic effect which is a solution consisting essentially of a pharmaceutically acceptable carrier having dissolved therein

a. an aminoalkyl benzoate local anesthetic compound in a concentration of from 0.05% to 5% by weight of the carrier and

b. a toxin selected from the group consisting of from 0.5 to 10 micrograms of tetrodotoxin per milliliter of the carrier and from 10 to 20 micrograms of desoxytetrodotoxin per milliliter of the carrier.

15. The method as defined by claim 14 wherein said component (b) is tetrodotoxin.

16. The method as defined by claim 14 wherein said component (b) is desoxytetrodotoxin.

17. The method as defined by claim 15 wherein the aminoalkyl benzoate is procaine.

18. The method as defined by claim 15 wherein the aminoalkyl benzoate is chloroprocaine.

19. The method as defined by claim 15 wherein the aminoalkyl benzoate is tetracaine.

20. The method as defined by claim 15 wherein the aminoalkyl benzoate is propoxycaine.

21. The method as defined by claim 15 wherein the aminoalkyl benzoate is hexylcaine.

22. The method as defined by claim 15 wherein the aminoalkyl benzoate is cyclomethycaine.

23. The method as defined by claim 15 wherein the aminoalkyl benzoate is benoxinate.

24. The method as defined by claim 15 wherein the aminoalkyl benzoate is butacaine.

25. The method as defined by claim 15 wherein the aminoalkyl benzoate is proparacaine.

26. The method as defined by claim 14 wherein said composition further contains an effective amount of a vasoconstrictor.

Description

The present invention relates to a novel anesthetic composition comprising a mixture of (1) tetrodotoxin or certain derivatives thereof and (2) another compound, generally a conventional local anesthetic compound, or a similar compound having nerve-blocking properties. The invention also relates to a process for preparing the novel anesthetic compositions and to their use for inducing anesthesia.

Toxins from marine sources of extraordinary potency have been known for many years. This application particularly concerns novel uses for tetrodotoxin.

Tetrodotoxin is obtained from the ovaries and eggs of several species of puffer fish of the suborder Gymnodontes. It is also found in certain species of California newts of the genus Taricha; and the toxin obtained from these species, known as tarichatoxin, is identical with tetrodotoxin. Tetrodotoxin has been purified, and its molecular structure is determined to be an amino perhydroquinazoline of the formula: ##STR1##

Tetrodotoxin and species in which it occurs are more fully described in Pharmacological Reviews, Vol. 18, No. 2, at pages 997-1049.

Experiments with isolated nerves have shown that tetrodotoxin behaves in a fundamentally different manner from local anesthetics such as procaine and cocaine. In a voltage-clamped giant axon from the squid or lobster, the latter agents reduce both inward initial sodium current and outward potassium current. With tetrodotoxin, however, inward sodium current can be reduced or even obliterated, while the outward potassium current is totally unaffected. There are few, if any, other substances in which this unique action has been established.

Tetrodotoxin has not found any practical use as an anesthetic. While the compound can be used to induce nerve blocks in laboratory animals, the anesthetic dose is slightly below the lethal dose, which precludes, as a practical matter the use of the compound as an anesthetic in its own right.

Quite surprisingly, combinations of tetrodotoxin with a local anesthetic compound have been found to possess unusual anesthetic properties. This is manifested most significantly in improved longevity of action of combinations of the toxin with local anesthetics. In these combinations, tetrodotoxin is used in concentrations below that which produces reliable nerve blocks, and well below the toxic level.

Investigation of a wide variety of local anesthetics has shown that the action of the foregoing toxin in increasing longevity of action is general. Local anesthetics may be classified by characteristic chemical type. Within each chemical type there may be unexplained variations of activity. However, in all cases investigated, each member of the groups investigated has behaved similarly when combined with the

foregoing toxin. Specific classes of local anesthetics investigated include anesthetic compounds characterized by

(i) the aminoacylanilide group, such as lidocaine, prilocaine, bupivacaine, mepivacaine and related local anesthetic compounds having various substituents on the ring system or amine nitrogen;

The following three ester types (ii), (iii) and (iv):

(ii) the aminoalkyl benzoate group, such as procaine, chloroprocaine, propoxycaine, hexylcaine, tetracaine, cyclomethycaine, benoxinate, butacaine, proparacaine, and related local anesthetic compounds;

(iii) cocaine and related local anesthetic compounds;

(iv) the amino carbamate group such as diperodon and related local anesthetic compounds;

(v) the N-phenylamidine group, such as phenacaine and related local anesthetic compounds;

(vi) the N-aminoalkyl amide group, such as dibucaine and related local anesthetic compounds;

(vii) the aminoketone group, such as falicain, dyclonine and related local anesthetic compounds; and

(viii) the aminoether group, such as pramoxine, dimethisoquine, and related local anesthetic compounds.

In each of the foregoing classes of local anesthetic compounds representative members have been enumerated. The experimental data support the conclusion that the observed effect of the toxin tested of unexpectedly extending the duration of action extend to the other known local anesthetic compounds of these groups and to the obvious modifications of the local anesthetic compounds tested. It may also be anticipated in the light of these discoveries that the novel combinations of the present invention will permit the use of concentrations of conventional local anesthetics in concentrations below the concentrations normally employed clinically. Thereby toxic manifestations sometimes observed as side effects can be minimized.

The chemical structures of some of the foregoing compounds are: ##STR2##

Other local anesthetic compounds which may be used in combination with tetrodotoxin (TTX) are the aminoacyl anilides described in the following table.

Table A

##STR3##

Compound	R	R.sup.1	R.sup.2	R.sup.3
A 2-tert. Butylamino-2',6'-acetoxylidide	H	H	H	C(CH.sub.3).sub.3
B 2-(N-n-Butyl-tert. butylamino)-2',6'-acetoxylidide	H	H	n-C.sub.4 H.sub.9	C(CH.sub.3).sub.3
C 2-(N-n-Propyl-tert. amylamino)-2',6'-acetoxylidide	H	H	n-C.sub.3 H.sub.7	C(CH.sub.3).sub.2 C.sub.2 H.sub.5
D 2-tert. Butylamino-2',6'-propionoxylidide	H	CH.sub.3	H	C(CH.sub.3).sub.3

E 2-(N-Ethyl-iso-propylamino)-2',6'-propionoxylidide	H	CH.sub.3	C.sub.2 H.sub.5	CH(CH.sub.3).sub.2
F 2-Methylamino-4'-(n-butoxy)-2',6'-dimethylpropion-anilide	n-C.sub.4 H.sub.9 O	CH.sub.3	H	CH.sub.3
G 2-(N-Methyl-n-propylamino)-2',6'-butyroxylidide	H	C.sub.2 H.sub.5	CH.sub.3	n-C.sub.3 H.sub.7
H 2-Dimethylamino-2',6'-acetoxylidide	H	H	CH.sub.3	CH.sub.3
J 2-Ethylamino-2',6'-acetoxylidide	H	H	H	C.sub.2 H.sub.5
K 2-Cyclobutylamino-2',6'- acetoxylidide	H	H	H	##STR4##
L 2-tert. Amylamino-2',6'-acetoxylidide	H	H	H	C(CH.sub.3).sub.2 C.sub.2 H.sub.5
M 2-(N-Methyl-n-butylamino)-2',6'-acetoxylidide	H	H	CH.sub.3	n-C.sub.4 H.sub.9
P 2-(N-Ethyl-sec. butylamino)-2',6'-acetoxylidide	H	H	C.sub.2 H.sub.5	CH(CH.sub.3)C.sub.2 H.sub.5
Q 2-Amino-2',6'-propionoxylidide	H	CH.sub.3	H	H
S 2-(N-Ethyl-n-propylamino)-2',6'-butyroxylidide	H	C.sub.2 H.sub.5	C.sub.2 H.sub.5	n-C.sub.3 H.sub.7
T 2-Diethylamino-2',6'-valeroxylidide	H	n-C.sub.3 H.sub.7	C.sub.2 H.sub.5	C.sub.2 H.sub.5

In the present invention the foregoing local anesthetics are used in a pharmaceutically acceptable carrier, such as water, water-ethanol, dextrose solutions, saline solution and blends thereof, in concentrations which are customarily used by physicians. Exemplary concentrations of local anesthetics having clinical application are:

	% by weight		
lidocaine	0.5	--	5
prilocaine	0.5	--	5
procaine	0.5	--	5
tetracaine	0.1	--	1
bupivacaine	0.25	--	1
hexylcaine	0.5	--	2.5
2-[N-n-propyl-tert. amylamino]-2',6'-acetoxylidide	0.1	--	2.0
2-[N-n-butyl-tert. butylamino]-			

2',6'-acetoxylidide	0.1	--	2.0

As mentioned above, the present invention also may permit the use of the usual local anesthetics in a lower than normal concentration. For example, the combination of tetrodotoxin with lidocaine permits the latter to be used in a concentration of as little as 0.05 percent by weight.

The carrier additionally contains from 0.5 to 10, usually from 0.5 to 5, micrograms per milliliter of tetrodotoxin or from 10 to 20 micrograms per milliliter of desoxytetrodotoxin. In addition, the local anesthetic preparation may contain a vascoconstrictor, as is well known in the art, such as epinephrine, norepinephrine, phenylephrine and levonordephrine.

The local anesthetic compositions may be prepared by dissolving the local anesthetic compound, tetrodotoxin or derivative thereof and a vascoconstrictor, when present, in the carrier or in separate portions of the carrier which are thereafter blended together.

Application of the local anesthetic compositions is accomplished in the usual manner, i.e., by infiltration or injection.

EXAMPLE 1

Female Charles River rats, weighing between 100 and 200 grams, were used. There were 5 rats per group and each animal received 0.2 milliliters of drug solution in the right thigh. The injections were made in such a way as to deposit the solution around the sciatic nerve trunk close to the popliteal space. After being injected, each animal was examined at intervals to determine onset, depth, and duration of nerve block as manifested by impairment of motor function in the injected leg. Frequencies of (a) complete block, (b) partial block, and (c) slight effect on motor function were noted for each group of animals. Two end points for duration of block were used: recovery of the ability to grasp when placed on an inclined screen and complete recovery of motor function.

All solutions contained 1 to 100,000 parts epinephrine which was added immediately prior to use. All solutions were freshly prepared on the day of use.

The results are summarized in Tables I- III. Depression was occasionally noted, but there were no fatalities with these doses of tetrodotoxin.

Table I: At 1 .mu.g/ml and 2 .mu.g/ml tetrodotoxin produced no complete blocks. At 5 .mu.g/ml, it produced complete blocks in all five injected. Mean onset time was about 20 minutes, and the blocks persisted for somewhere between 51/2 hours and 24 hours. All animals were completely recovered when examined 22 to 24 hours post injection. Because this concentration of tetrodotoxin by itself produced 100 percent frequency and blocks of such long duration, there are no differences, except in onset times, between the results obtained with it alone and those obtained with the tetrodotoxin-lidocaine combination. However, the combinations of 1 .mu.g/ml and 2 .mu.g/ml of tetrodotoxin with lidocaine clearly show durations of block that are markedly greater than those obtained with lidocaine alone.

TABLE I

RAT SCIATIC NERVE BLOCKS

Compound	Concentration as Base	pH	Onset (min.)	Frequency C	Frequency P	Frequency S	Duration (min.) Mean .+-. S. D. C.R.	Duration (min.) Mean .+-. S. D. R.G.

Tetrodotoxin								
	1 .mu.g/ml	4.4	--	0/5	1/5	4/5	--	--
	2 .mu.g/ml	5.4	--	0/5	2/5	3/5	--	--
	5 .mu.g/ml	4.3	22	5/5	--	--	--	5.5<24 hrs
Lidocaine								
	0.125%	5.1	8	5/5	--	--	85 .+-. 2	84 .+-. 1.5
	0.25%	5.0	5	5/5	--	--	108 .+-. 22	99 .+-. 24
Combinations								
T/L	1/0.125	4.9	5.5	5/5	--	--	309 .+-. 17	251 .+-. 51
T/L	2/0.125	4.8	5.0	4/5	1/5	--	316 .+-. 33	290 .+-. 46
T/L	5/0.125	4.6	3.5	5/5	--	--	--	6<24 hrs.
T/L	1/0.25	4.7	4.5	5/5	--	--	5.5<24 hrs.	299 (2)
T/L	2/0.25	4.8	3.0	5/5	--	--	--	5.5<24 hrs.
T/L	5/0.25	4.6	1.5	5/5	--	--	--	6<24 hrs.

NOTES: C = Complete block; P = Partial block; S = Slight effect; R.G. = Recovery of grasping; C.R. = Complete Recovery; T = Tetrodotoxin; L = Lidocaine. Durations are for complete blocks only. Onset times are approximate. The pH's are after addition of epinephrine; all solutions contained 1:100,000 epinephrine. Numbers of blocks are in specific instances shown in parentheses.

Table II: As in the first study, 1 .mu.g/ml of tetrodotoxin produced no complete blocks; however, 2 .mu.g/ml produced a complete block, with a duration of about 2 hours, in one out of five injections. The frequency of block with 3 .mu.g/ml was only two out of five, but the block persisted for between 5 and 24 hours. In this study lower concentrations of lidocaine were used in order to ascertain whether or not the combinations show better frequencies than either tetrodotoxin or lidocaine alone.

TABLE II

RAT SCIATIC NERVE BLOCKS

Compound	Concentration as Base	pH	Onset (min.)	Frequency C	Frequency P	Frequency S	Duration (min.) Mean .+-. S. D. C.R.	Duration (min.) Mean .+-. S. D. R.G.
Tetrodotoxin								
	1 .mu.g/ml	4.6	--	0/5	0/5	5/5	--	--
	2 .mu.g/ml	4.7	31	1/5	0/5	4/5	120	102
	3 .mu.g/ml	4.5	56	2/5	0/5	3/5	--	5<24 hrs.
Lidocaine								
	0.05%	4.6	--	0/5	2/5	3/5	--	--
	0.1%	4.6	43	2/5	2/5	1/5	58	44
Combinations								
T/L	1/0.05	4.6	31	1/5	2/5	2/5	68	48
T/L	2/0.05	4.4	10	2/5	2/5	1/5	255	176
T/L	3/0.05	4.5	16	3/5	2/5	--	6 1/2<24 hrs.	359 .+-. 42
T/L	1/0.1	4.5	11	2/5	3/5	--	144	93
T/L	2/0.1	4.6	6	4/5	0/5	1/5	242 .+-. 68	188 .+-. 83
T/L	3/0.1	5.2	14	4/5	1/5	--	304 (1)	317 .+-. 50

6 1/2<24 hrs.
(3)

See notes under Table I.

Table III: Tetrodotoxin at 3 .mu.g/ml produced in three out of five animal blocks that lasted between 4 and 24 hours. In combinations with several local anesthetic agents, frequency was improved and onset times were shorter than with tetrodotoxin alone. All the combinations containing 1 .mu.g/ml of tetrodotoxin exhibited durations of block much greater than obtained with the local anesthetic agents alone. The study clearly demonstrates that, in rat sciatic nerve blocks, the presence of concentrations of tetrodotoxin that by themselves are subthreshhold can cause marked increases in the durations of block of several local anesthetic agents.

TABLE III

RAT SCIATIC NERVE BLOCKS

Compound	Concentration as base	pH	Onset (min.)	Frequency C.	Frequency P	Frequency S	Duration (min.) Mean .+-. S. D. C.R.	Duration (min.) Mean .+-. S. D. R.G.
Tetrodotoxin	1 .mu.g/ml	4.6	--	0/5	1/5	4/5	--	--
	3 .mu.g/m.	4.3	48	3/5	2/5	--	4<24 hrs.	
Lidocaine	2.0%	4.4	2.0	5/5	--	--	172 .+-. 17	160 .+-. 12
T/L	1/2.0	4.5	1.5	5/5	--	--	223(2) 4 1/2<24 hrs. (3)	188(2)
T/L	3/2.0	4.3	1.5	5/5	--	--	4 1/2<24 hrs.	
Bupivacaine	0.5%	5.0	2.5	5/5	--	--	232 .+-. 39	183 .+-. 18
T/B	1/0.5	5.2	6.0	5/5	--	--	282 (2) 5<24 hrs. (3)	265 .+-. 45
T/B	3/0.5	5.4	2.5	5/5	--	--	5<24 hrs.	
Prilocaine	2.0%	5.0						

2.5 5/5
-- -- 153 .+-. 16
123 .+-. 6
T/Pr 1/2.0 4.8
<1.0
5/5
-- -- 5<24 hrs.
251 .+-. 26
T/Pr 3/2.0 4.8
2.0 5/5
-- -- 5<24 hrs.
Tetracaine
0.25% 5.2
4.0 3/5
2/5
-- 206(2) 180(2)
4 1/2<24 hrs.
(1)
T/Tet 1/0.25 5.9
5.5 4/5
1/5
-- 4<24 hrs.
T/Tet 3/0.25 6.4
5.0 5/5
-- -- 3<24 hrs.

See Notes under Table I.
B = Bupivacaine; Pr = Prilocaine; Tet = Tetracaine.

EXAMPLE 2

The use of anesthetics of the present invention is also shown through peridural blocks in the cat. The surgical techniques and testing methods have been described in detail (Duce et al: Brit. J. Anaesth., Vol. 41, 579-587 (1969)). The animals were treated according to the following scheme in this study:

Cat No.	Weight and Sex	Day (treatment) 1	2	3	4	5
124	3.6 kg F	X	L	TTX	L/TTX	X
125	2.8 kg F	X	L	L/TTX	TTX	X
127	4.0 kg M	X	TTX	L	L/TTX	X
128	2.8 kg F	X	TTX	L/TTX	L	X

X = XylocaineO.sup.R HCl, 2% as salt
L = Lidocaine HCl, 2% as base
TTX = Tetrodotoxin, 1 .mu.g/ml
F = female
M = male

All animals were tested with 2 percent Xylocaine (a commercial local anesthetic composition based on lidocaine as the active ingredient) on Days 1 and 5 to ascertain the stability of the peridural cat preparation. Within the test period, laboratory-prepared samples of lidocaine were used containing only lidocaine and epinephrine or lidocaine, epinephrine and tetrodotoxin in specified proportions. Solutions were freshly prepared each day of use; epinephrine was added and the pH taken shortly before administration. The pH's of the solutions were: tetrodotoxin, 4.5-6.75; lidocaine HCl, 4.75-4.8; lidocaine/tetrodotoxin, 4.75-4.9.

The results are summarized in Table IV. In general, no overt systemic effects were noted following administration of the test solutions. Animal No. 127 exhibited salivation and emesis, with bile present, about 3 hours and 45 minutes after administration of the lidocaine/tetrodotoxin combination. However, these observations were not considered significant.

Statistical analysis of the data showed that the Xylocaine.RTM. control values obtained on days 1 and 5 are not significantly different. Since tetrodotoxin alone produced no blocks, it as excluded from the analysis of variance in order to keep the variance reasonably homogeneous. A four-way analysis of variance was, therefore, done only with the data obtained with 2 percent lidocaine and with the lidocaine/tetrodotoxin combination. The durations of block with the lidocaine tetrodotoxin combination were statistically significantly longer than with lidocaine itself.

TABLE IV

PERIDURAL ANESTHESIA IN CAT

Compound and Concentration	Deep Motor Block Duration x .+-. S.E.	Onset	Frequency	Block of Support of Weight Duration x .+-. S.E.	Onset	Frequency	Block of Flexion Reflex Duration x .+-. S.E.	Onset	Frequency
2% XylocaineO .sup.R (Day 1)	119 .+-. 12	1	8/8	98 .+-. 13	<1	8/8	48 .+-. 10	8	4/8
2% XylocaineO .sup.R (Day 5)	124 .+-. 11	1-2	8/8	106 .+-. 9	1	8/8	70 .+-. 17	7	6/8
1 .mu.g/ml Tetrodotoxin	--	--	0/8	--	--	0/8	--	--	0/8
2% Lidocaine	115 .+-. 10	2-3	8/8	88 .+-.10	2	8/8	66 .+-. 19	7	5/8
Lidocaine/tetrodotoxin	226 .+-. 9	<1	8/8	188 .+-. 9	<1	8/8	109 .+-. 8	7	7/8

Durations are in minutes.
Mean onset times are approximate.
Durations of block of flexion reflex in this table were calculated without zero values.
All solutions contained 1:100,000 epinephrine.

EXAMPLE 3

The effectiveness of the local anesthetics of the present invention in the absence of epinephrine is shown by the data set forth in Tables V, VI and VII. The data summarized in these tables were obtained following the same procedures as described in Example 1.

Five separate studies were done, and a tetrodotoxin control group was run in each study. Frequency of block with tetrodotoxin ranged from 0/5 to 3/5, and durations ranged from about 180 to 240 minutes.

Frequency of block was 5/5 with all combinations except that containing phenacaine (Table VI). The one partial block in this case may have been due to failure to inject the solution sufficiently close to the sciatic nerve trunk. The frequency of block with the diperodon-tetrodotoxin, cyclomethycaine-tetrodotoxin and dibucaine-tetrodotoxin were better than with diperodon, cyclomethycaine, dibucaine or tetrodotoxin by itself.

In all cases, the combinations produced durations markedly longer than obtained with the local anesthetics alone. The durations of tetrodotoxin alone were closer to those with the combinations; the frequencies were consistently lower than those produced by the combinations.

TABLE V

RAT SCIATIC NERVE BLOCKS

Compound	% Conc. as base	pH	Onset (min.)	Frequency C	Frequency P	Duration (min.) Mean .+-. S. D. C.R.	Duration (min.) Mean .+-. S. D. R.G.
Lidocaine	2.0	4.8	1	5/5	--	108 .+-. 48	103 .+-. 37
Lidocaine/TTX	2.0	4.8	<1	5/5	--	385 .+-. 25	348 .+-. 17
Procaine	2.0	5.5	2.5	5/5	--	62 .+-. 7	60 .+-. 7
Procaine/TTX	2.0	5.5	2	5/5	--	356 .+-. 53	314 .+-. 58
Chloroprocaine	2.0	5.4	1.5	5/5	--	111 .+-. 65	87 .+-. 36
Chloroprocaine/TTX	2.0	5.3	1	5/5	--	351 .+-. 73	325 .+-. 46
Tetrodotoxin	2.mu.g/ml	6.0	13	2/5	0/5	242 .+-. 4	226.+-. 5
Diperodon	0.25	5.4	22	2/5	0/5	124	64
Diperodon/TTX	0.25	5.4	9	5/5	--	332 .+-. 59	286 .+-. 33

Propoxycaine	0.25	5.4	4	5/5	--	64 .+-. 12	52 .+-. 15
Propoxycaine/TTX	0.25	5.5	1.5	5/5	--	262 .+-. 91	239 .+-. 102
Hexylcaine	0.5	5.6	3	5/5	--	112 .+-. 12	99 .+-. 17
Hexylcaine/TTX	0.5	5.5	4.5	5/5	--	339 .+-. 17	303 .+-. 12
Cocaine	0.25	6.1	4.5	5/5	--	98 .+-. 9	86 .+-. 17
Cocaine/TTX	0.25	5.6	5	5/5	--	(1 day)	361 .+-. 28
Tetrodotoxin	2.mu.g/ml	6.1	16	2/5	1/5	216 .+-. 51	161

TTX = Tetrodotoxin, 2 .mu.g/ml: C = Complete block; P = Partial block; C.R. = Complete recovery of normal motor function; R.G. = Recovery of grasping; Durations are for complete blocks only; Onset times are approximate.

TABLE VI

RAT SCIATIC NERVE BLOCKS

Compound	% Conc. as base	pH	Onset (min.)	Frequency C	Frequency P	Duration (min.) Mean .+-. S. D. C.R.	Duration (min.) Mean .+-. S. D. R.G.
Phenacaine	0.25	5.6	4.5	5/5	--	78 .+-. 32	70 .+-. 32
Phenacaine/TTX	0.25	5.5	8	4/5	1/5	282 .+-. 74	253 .+-. 71
Benoxinate	0.25						

		5.6	3	5/5	--	116 .+-. 24	97 .+-. 20
Benoxinate/TTX	0.25	5.6	8	5/5	--	320 .+-. 54	285 .+-. 58
Butacaine	0.25	5.8	6	4/5	1/5	73 .+-. 7	67 .+-. 2
Butacaine/TTX	0.25	5.6	5	5/5	--	241 .+-. 24	204 .+-. 37
Tetrodotoxin	2 .mu.g/ml	6.1	18	1/5	--	181	150
Proparacaine	0.5	6.0	1.5	5/5	--	98 .+-. 20	89 .+-. 14*
Proparacaine/TTX	0.5	6.1	2	5/5	--	429 .+-. 41	415 .+-. 50*
Tetrodotoxin	2.mu.g/ml	6.2	13	3/5	2/5	222 .+-. 48	206 .+-. 42

TTX = Tetrodotoxin, 2.mu.g/ml; C = Complete block; P = Partial block; C.R = Complete recovery of normal motor function R.G. = Recovery of grasping; Durations are for complete blocks only; Onset times are approximate.
*Means of 3 animals; 2/5 died.

TABLE VII

RAT SCIATIC NERVE BLOCKS

Compound	% Conc. as base	pH	Onset (min.)	Frequency C	Frequency P	Duration (min.) Mean .+-. S. D. C.R.	Duration (min.) Mean .+-. S. D. R.G.
Cyclomethycaine	0.125	5.1	17	1/5	4/5	145	115
Cyclomethycaine/TTX							

	0.125	5.2	5	5/5	--	273 .+-. 43	231 .+-. 41
Dibucaine	0.125	5.4	6	3/5	1/5	125 .+-. 22	108 .+-. 25
Dibucaine/TTX	0.125	5.4	6	5/5	--	324 .+-. 47	272 .+-. 56
Tetrodotoxin	2.mu.g/ml	5.6	--	0.5	1/5	--	--

TTX = Tetrodotoxin, 2.mu.g/ml; C = Complete block; P = Partial block; C.R = Complete recovery of normal motor function; R.G. = Recovery of grasping
Durations are for complete blocks only;
Onset times are approximate.

EXAMPLE 4

The use of desoxytetrodotoxin was tested following the procedure described in Example 1. The desoxy derivative was substituted for the tetrodotoxin referred to in Example 1. Desoxytetrodotoxin was tested, without epinephrine, in rat sciatic nerve blocks. At concentrations of 5, 10 and 20 .mu.g/ml it produced no blocks. The duration of block of a combination containing 2 percent lidocaine and 5 .mu.g/ml of desoxytetrodotoxin was not significantly different from that of 2 percent lidocaine alone. However, combinations containing 10 and 20 .mu.g/ml of desoxytetrodotoxin produced blocks that were significantly longer (1.4-1.6 times) than that of lidocaine alone ($0.008 > p > 0.016$).

This result is to be expected based on the tests of tetrodotoxin in view of the lower activity shown by the desoxy derivative in toxicity tests. Literature on the toxicity of tetrodotoxin and its desoxy derivative reports the latter to be between one quarter and one tenth as toxic as its parent toxin.

EXAMPLE 5

Method: Mature male beagles are surgically prepared by implantation of a cannula into a lumbar vertebra so that drug solutions may be administered into the peridural space. After administration of local anesthetic solutions, the animals are examined at intervals for duration of loss of pain in the scrotal area and in the digits of the hind limbs as well as for loss of ability to support their weight.

Response to and awareness of pain stimuli in scrotal areas is a test for anesthetic block in spinal roots L3-4 and Sl-2-3. These roots are the furthest removed from the point of injection (L6) and, therefore, least likely to be affected by the anesthetic. Return of response to pain in the scrotum is often the first sign of recovery and indicates recession of anesthesia to at least L4 anteriorly and S2 posteriorly.

TABLE VIII

PERIDURAL ANESTHESIA IN DOGS
Onset: mean and range
Duration: mean and range
Compound and

Concentration	Digital Pain	Scrotal Pain	Weight Support	Digital Pain	Scrotal Pain	Weight Support
Lidocaine 2% (n=3)	7	8	<5	127 76-162	111 62-152	137 108-162
Tetrodotoxin 4 .mu.g/ml (n=2)	19.5 19-20	15*	<17	225 87-350	0-125	406 339-473
Lidocaine 2% Tetrodotoxin 4 .mu.g/ml (n=2)	<5	<5	<3	316 245-387	301 235-367	462 400-525

*One animal only; no anesthesia in second animal.
Onsets and durations are in minutes.
All Solutions contained 1:100,000 epinephrine.
Volume of administration = 5 ml.
n = number of animals
NOTE:
(1) With lidocaine onset is rapid, frequency of block is 100%, but durations are short.
(2) With tetrodotoxin durations are long, but onset is slow and frequency of block of scrotal pain is poor.
(3) With the combination onset is rapid, frequency is 100% and durations are long.

EXAMPLE 6

Following the method described in Example 1 above, various local anesthetic compounds alone, TTX alone and combinations of the compounds with TTX were tested for their ability to block the rat sciatic nerve. TTX was used uniformly in the amount of 2 .mu.g/ml. Each of the compositions tested contained epinephrine in concentration of 1:100,000. The results are presented in Table IX. In the case of compound A in 0.5% concentration, duration was about 126 minutes. TTX alone was about 295 minutes but frequency was not good. In combination, frequency was good and duration was greater than 420 minutes.

In the case of compound D at 0.25% concentration, duration was about 128 minutes alone but greater than 420 minutes in combination with TTX. In the case of compound E at 0.25% concentration, no blocks were observed alone, but in combination with TTX the duration was about 148 minutes. In the case of compound F alone at 0.125% concentration, duration was only 78 minutes with poor frequency, whereas in combination with TTX duration was greater than 322 minutes and frequency had improved. For compound G at 0.5% concentration, duration was 104 minutes alone and about 286 minutes in combination with TTX.

It should be noted moreover that in the case of TTX alone, the frequency and duration were quite variable ranging from zero frequency to 4 out of 5, and ranging from zero duration to 295 minutes or more.

Table IX

Rat Sciatic Nerve Blocks
Tetrodotoxin (TTX) (2 .mu.g/ml) and Various Local Anaesthetic Compounds. Epinephrine concentration 1:100,000.

Compound	Frequency	Duration (min.) Mean .+-. S.D.
TTX	2/5	295*
A (0.5%)	5/5	126 .+-. 12
TTX + A (0.5%)	5/5	>420, <24 hrs.**
A (1.0%)	5/5	157 .+-. 18
TTX + A (1.0%)	5/5	>420, <24 hrs.
TTX	4/5	316 .+-. 10*
D (0.25%)	5/5	128 .+-. 13
TTX + D (0.25%)	5/5	>420, <24 hrs.
D (0.5%)	5/5	133 .+-. 7
TTX + D (0.5%)	5/5	>420, <24 hrs.
TTX	0/6	0
E (0.25%)	0/6	0
TTX + E (0.25%)	4/6	148 .+-. 27
TTX	0/5	0
F (0.125%)	1/5	78
TTX + F(0.125%)	3/5	>322 min.
TTX	0/5	0
G (0.5%)	5/5	104 .+-. 14
TTX + G (0.5%)	4/5	286 .+-. 197

* One animal blocked >420 min.
** >420, <24 hrs. means that the animals returned to normal during a period when they were not observed, this period being longer than 7 hrs. and shorter than 24 hrs.

EXAMPLE 7

In vitro tests were made on the isolated intact frog sciatic nerve using compounds B, C and lidocaine alone and in combination with TTX. The results and the method followed are presented in Table X. The reduction in the action potential of compound B alone was 22% and for TTX alone it was 15%, as compared with a reduction of 94% for the combination. For compound C alone the reduction was 24%, and for TTX alone 29%. whereas the combination again reduced the potential by 94%. For lidocaine and TTX each alone the reductions were 15% and 7%, respectively, as compared with a reduction of 61% for the combination of the two.

Table X

Block of Isolated Intact Frog Sciatic Nerve.

Compound	pH	Concn. mM	Percent reduction of the action potential. Mean and range	Number of experiments
B	5.6	0.625	22 (10-38)	16
TTX	5.6	3.10.sup.-.sup.4	15 (8-)	17
B + TTX	5.6	as above	94 (80-100)	17
C	5.6	0.156	24 (15-52)	8
TTX	5.6	3.10.sup.-.sup.4		

			29 (14-80*)	6
C + TTX	5.6	as above	94 (78-100)	12
Lidocaine				
	7.0	0.625	15 (6-30)	6
TTX	7.0	1.10.sup.-.sup.4		
			7 (2-12)	6
Lidocaine)				
)	7.0	as above	61 (20-100)	12
+ TTX)				

*Occasionally a high value is observed, probably caused by a minute damag to the nerve sheath during dissection. It takes about 50 times the concentration of TTX which is necessary to block a desheathed nerve in order to obtain the same degree of block of an intact (sheathed) nerve. as

Method: The method is essentially is described by A. P. Truant, Arch. Int. Pharmacodyn. 115, 483-497 (1958).

Sciatic nerve trunks of Rana pipiens are prepared by dissecting the nerve from its roots in the spinal cord to the ankle and placing it on silver-silver chloride electrodes so that stimulation and recording of the action potential can be performed during the course of application of the test compounds and during the recovery period. The bathing solution is Tasaki Ringer's. The observations lasted for 40 minutes allowing the action potentials to reach essentially a stable value (equilibrium).

EXAMPLE 8

Using the procedure described in Example 1 above, the effect of several known vasoconstrictors on rat sciatic nerve blocks was investigated using lidocaine (0.125%) and tetrodotoxin (2 .mu.g/ml) in combination. The results are given in Table XI. Without any vasoconstrictors, the frequency was very poor and the duration of block was 174 minutes. With phenylephrine, levonordefrin, or epinephrine, however, frequency was greatly improved and duration had about doubled.

Table XI

Effect of Vasoconstrictors on Rat Sciatic Nerve Blocks Obtained with Lidocaine (0.125%) and Tetrodotoxin (2 .mu.g/ml).

Vasoconstrictor	Concn.	Frequency	Duration of Block (min.) Mean .+-. S. D.
None	--	1/5	174
Phenylephrine	1:20,000	5/5	377 .+-. 27
Levonordefrin	1:20,000	5/5	354 .+-. 12
Epinephrine	1:200,000	5/5	368 .+-. 24

EXAMPLE 8a

Using the procedure described in Example 1, except that no epinephrine was added to the solutions tested, the local anesthetics falicain and pramoxine were tested for blockage on the rat sciatic nerve alone and in combination with TTX at 2 .mu.g/ml. The results are presented in the following Table XII.

TABLE XII

Rat Sciatic Nerve Blocks

	Frequency	Duration
0.25% falicain	5/5	55 .+-. 22
0.25% falicain .+-. TTX, 2 .mu.g/ml	5/5	116 .+-. 71
0.25% pramoxine	0/5	0
0.25% pramoxine .+-. TTX, .mu.g/ml	2/5	190 .+-. 76
TTX, 2 .mu.g/ml	0.5	0

It will be observed that the ingredients were tested at dose level that did not result in any anesthesia at all for two of them, and only 55 min. for the third one, whereas the combination gave anesthesia about 2 to 3 hrs. The frequency of complete block was raised from 0 to 40% in the case of pramoxine.

Compounds A, B, C, D and L described in Table A above are made by the procedure described in U.S. patent application Ser. No. 369,146, filed June 12, 1973, which is a continuation-in-part of Ser. No. 325,378, filed January 22, 1973, now abandoned, both assigned to the same assignee as the present application, which disclosure is incorporated herein by reference.

The method of preparing compounds S and T is disclosed in U.S. patent application Ser. No. 164,022 filed July 19, 1971, now U.S. Pat. No. 3,812,147, which is incorporated herein by reference.

The method of preparing compound Q is disclosed in U.S. patent application Ser. No. 321,590 filed January 8, 1973, now abandoned, which is incorporated herein by reference.

Compounds H, J and M and mepivacaine are known compounds disclosed in the published literature.

EXAMPLE 9

Synthesis of 2-(N-ethyl-isopropylamino)-2',6'-propionoxylidide (Compound E)

A mixture of 12.81 g (0.050 mole) of 2-bromo-2',6' -propionoxylidide, 11.31 g (0.130 mole) ethyl-isopropylamine and 30 ml dry toluene was heated in a glass-lined, stainless-steel pressure vessel at 105.degree. for 20 hours. After cooling to 25.degree., the brown reaction mixture was filtered, extracted three times with a total of 50 ml of 3 N HCl. The aqueous solution was heated to 75.degree. for ten minutes with decolorizing carbon and then filtered. To the chilled solution was added 10 ml concentrated NH.sub.3. The product which precipitated was filtered, washed, and dried. Yield: 6.93 g (52.9%) m.p. 50-2.degree..

Anhydrous ethereal HCl was added to 6.90 g of the above base dissolved in 100 ml dry ether until the solution was acidic to moist pH paper, giving 6.15 g of tacky brown material, m.p. 191.degree.-201.degree.. The hydrochloride was recrystallized from a mixture of butanone and alcohol. Yield: 6.02 g, m.p. 207.5.degree. - 209.degree..

Analysis: Calc'd. for C.sub.16 H.sub.27 ClN.sub.2 O: C 64.30, H 9.11, N 9.37, Cl 11.86. Found: C 64.16; H 9.16, N 9.49, Cl 12.09.

EXAMPLE 10

A. Synthesis of 2-Bromo-4'-butoxy-2',6'-dimethyl propionanilide

To a chilled (ca 10.degree.) solution of 50.7 g (0.263 mole) of 4-butoxy-2,6-dimethylaniline [Buchi et al., Helv. Chim. Acta, 34, 278 (1951)] in 224 ml glacial acetic acid was added rapidly 62.4 g (.289 mole) of 2-bromo-propionyl bromide and immediately thereafter a chilled (ca 5.degree.) solution of 87.2 g sodium acetate trihydrate in 362 ml water. This mixture was shaken for 1/2 hour, filtered, washed

with water until the washes were neutral, and dried in vacuo over silica gel and KOH; yield 68.9 g (71.6%); m.p. 132.5.degree. - 133.5.degree.. The product was recrystallized from 95% ethanol; m.p. 135.5.degree. - 136.degree..

Analysis: Calc'd for C.sub.15 H.sub.22 NO.sub.2 Br : C 54.87, H 6.76, Br 24.34. Found:C55.06, H 6.22, Br 24.69.

B. Synthesis of 2-Methylamino-4'-butoxy-2',6'-dimethyl-propionanilide (Compound F)

To a cold stirred solution of 14.8 g. of monomethyl amine in 250 ml dry benzene was added (portionwise, keeping temperature below 10.degree.) 19.5 g (0.0594 mole) of 2-bromo-4'-butoxy-2',6'-dimethyl propionanilide (made according to the procedure in the first part of this example); this dissolved readily forming a clear solution. The mixture was heated to 70.degree. for ca 1 hr. with stirring, at which point a white precipitate had separated and reflux became so vigorous that the reaction had to be controlled by external cooling.

The precipitated methylammonium bromide was filtered off. Excess amine and solvent were removed in vacuo from the filtrate, giving a white residue which was dissolved in 120 ml 0.5 M HCl and filtered. The filtrate was extracted with 3 .times. 25 ml. ether; and the ether extracts discarded.

The aqueous phase was alkalized to pH 11, and extracted with ether; the combined extracts were dried (Na.sub.2 SO.sub.4), filtered, and evaporated, giving a yield of 8.7 g (52.7%); m.p. 107.degree.-107.5.degree.. Recrystallization from cyclohexane did not affect the melting point.

Analysis: Calc'd. for C.sub.16 H.sub.26 N.sub.2 O.sub.2 : C 69.0; H 9.41; N 10.06. Found: C 69.0; H 9.17; N 10.06.

EXAMPLE 11

Synthesis of 2-(N-Methyl-n-propylamino)-2',6'-butyroxylidide (Compound G)

To a stirred solution of N-methyl-n-propylamine (9.10 g, 0.125 mole) in 175 ml of anhydrous benzene was added 2-iodo-butyro-2',6'-xylidide (13.2 g, 0.0415 mole). The mixture was allowed to reflux for 5 hrs.

The reaction mixture was extracted with 1 M HCl. After filtration to remove trace insolubles, the pH was adjusted to 9 with 7 M NaOH, which caused the formation of a light-yellow waxy solid. The latter was filtered, washed with water, and dried; yield 4.00 g (36.7%).

This base was converted to the hydrochloride salt with ethereal HCl. The hydrochloride was twice-recrystallized from ethanol/ether, affording crystals melting at 214.degree.-215.degree. C.

Analysis: Calc'd. for C.sub.16 H.sub.27 ClN.sub.2 O : C 64.3; H9.11; Cl 11.86. Found: C 64.4; H 9.01; Cl 11.80.

EXAMPLE 12

Synthesis of 2-Cyclobutylamino-2',6'-acetoxylidide (Compound K).

To a solution of cyclobutylamine (39.8 g) in 600 ml benzene was added 2-chloro-2',6'-acetoxylidide (49.4 g), slowly, with stirring, and the mixture was refluxed for about 5 hrs. After cooling, the mixture was filtered to remove the cyclobutylammonium chloride formed. The filtrate was stripped of solvent and excess amine in vacuo; leaving a crude residue.

The residue was dissolved in 0.5 M hydrochloric acid, the solution was made alkaline with sodium hydroxide solution and the base was extracted carefully with ether. The ether solution was dried (Na.sub.2 SO.sub.4), the ether and low-boiling components were evaporated in vacuo at

40.degree.-50.degree. C. and the residue converted to a hydrochloride by addition of ethereal hydrogen chloride to its filtered ether solution. From the hydrochloride the base was obtained by dissolution in water, addition of sodium hydroxide solution to alkaline pH, extraction with ether, drying of the ether extract (Na.sub.2 SO.sub.4), filtering, and evaporation of the ether. The base could be recrystallized from cyclohexane, petroleum ether (b.p. 60.degree.-110.degree. C.), or heptane. The melting point was found to be 75.degree.-78.degree. C.

Analysis: Calc'd. for C.sub.14 H.sub.20 N.sub.2 O : C 72.4, H 8.68, N 12.06. Found: C 72.4, H 8.88, N 11.93.

EXAMPLE 13

A. Synthesis of 2-(sec-butylamino)-2',6'-acetoxylidide

To a solution of 62.2 g of sec-butylamine in 500 ml benzene was added slowly 41.5 g of 2-chloro-2',6'-acetoxylidide. The mixture was heated to reflux for seven hours and allowed to cool overnight. The precipitate of sec-butyl amine hydrochloride that formed was filtered off and the filtrate was evaporated to an oily residue. The residue was dissolved in ether, and the solution was filtered, dried (Na.sub.2 SO.sub.4), and evaporated to an oily residue (45.7 g). This crude product was distilled under vacuum, giving an oily liquid that solidified when chilled. Yield: 38.5 g (78%); b.p. 146.degree./0.05 mm; m.p. 44.5.degree.-45.5.degree..

Analysis: Calc'd. for C.sub.14 H.sub.22 N.sub.2 O : C 71.75, H 9.46, N 11.96. Found: C 71.99, H 9.35, N 12.12. The hydrochloride melted at 176.5 - 178.5.degree.. ##STR5##

B. Synthesis of 2-(N-ethyl-sec-butylamino)-2',6'-acetoxylidide (Compound P)

To 140 g of diethyl sulfate was added 30.5 g of 2-(sec-butylamino)-2',6'-acetoxylidide (made by the method described in the first part of this example). The mixture was heated to 100.degree.-110.degree. for five hours and cooled. Water and 5 N HCl were added to pH 2, forming a second phase. After stirring, the aqueous phase (pH 2) was separated, washed with two 100 ml portions of ether and brought up to pH 9 with concentrated NH.sub.3. The basic aqueous phase was extracted with five 100 ml portions of ether. The solvent was stripped in vacuo from the combined ether phases, leaving a solidifying oil which was dissolved in ether, dried (Na.sub.2 SO.sub.4), filtered, and evaporated in vacuo. Yield: 26.2 g (76.8%); m.p. 50.5.degree. - 54.5.degree.. The product was twice distilled under high vacuum : b.p. 147.degree./0.025 mm; 165.degree./0.4 mm. Yield of redistilled product: 21.4 g (62.7%).

Analysis: Calc'd. for C.sub.16 H.sub.26 N.sub.2 O : C 73.23%, H 9.99%, N 10.68%. Found: C 73.06%, H 9.66%, N 10.47%.

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(1 of 1)

United States Patent ***6,030,974***
Schwartz , et al. **February 29, 2000**

Method of anesthesia

Abstract

A method of producing local anesthesia in a mammal experiencing pain in an epithelial tissue region is described. The method includes topically administering to the region, in a suitable pharmaceutical vehicle, an effective dose of a long-acting sodium channel blocking compound.

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Field of Search: 514/267,817,818,912

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Parent Case Text

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/040,903, filed Apr. 2, 1997 and U.S. Provisional Application No. 60/076,317, filed Feb. 27, 1998.

Claims

1. A method of producing local anesthesia in a partially or completely de-epithelialized tissue region of a mammal, comprising

topically administering an anesthetically effective dose of a pharmaceutical composition consisting essentially of a long-acting sodium channel blocking compound, in a pharmaceutically suitable vehicle, to said de-epithelialized tissue region of said mammal, wherein said de-epithelialized tissue region is a corneal region, a region in the upper or lower gastrointestinal tract, or a genital lesion in the genital area.

2. The method of claim 1, wherein said long-acting sodium channel blocking compound does not inhibit re-epithelialization of said epithelial tissue region.

3. The method of claim 2, wherein said sodium channel blocking compound is administered every 6-8 hours for between about 24-72 hours.

4. The method of claim 2, wherein said long-acting sodium channel blocking compound is a compound capable of specifically binding to a site on an extracellular region of a sodium channel alpha subunit.

5. The method of claim 4, wherein said site is on an SS2 extracellular region of a sodium channel alpha subunit.

6. The method of claim 5, wherein said long-acting sodium channel blocking compound is tetrodotoxin.

7. The method of claim 6, wherein said effective dose of tetrodotoxin is administered from a formulation containing tetrodotoxin at a concentration of between 0.001-10 mM.

8. The method of claim 6, wherein said tetrodotoxin is administered in a vehicle having a pH of between 4-8.

9. The method of claim 8, wherein said vehicle has a pH of between 5-7.5.

10. The method of claim 5, wherein said long-acting sodium channel blocking compound is saxitoxin.

11. The method of claim 1, wherein said administering is to a partially or completely de-epithelialized comeal tissue region.

12. The method of claim 1, wherein said administering comprises instilling drops of said sodium channel blocking compound to the eye following corneal surgery.

13. The method of claim 1, wherein said epithelial tissue region is a region in the upper or lower gastrointestinal tract.

14. The method of claim 1, wherein said epithelial tissue region is associated with genital lesions in the genital area.

15. A method of producing local anesthesia in an eye of a mammal, comprising

topically administering to the corneal surface of the eye of said mammal, in an ophthalmically suitable vehicle, an anesthetically effective dose of a pharmaceutical composition consisting essentially of a long-acting sodium channel blocking compound, said corneal surface having an epithelial layer that is partially or completely de-epithelialized.

16. The method of claim 15, wherein said long-acting sodium channel blocking compound is a compound capable of specifically binding to a site on an extracellular region of a sodium channel alpha subunit, wherein said site is on either an SS1 region or an SS2 region.

17. The method of claim 16, wherein said long-acting sodium channel blocking compound is tetrodotoxin and said effective dose is administered from a formulation containing tetrodotoxin at a concentration between about 0.001-10 mM.

18. The method of claim 17, wherein said long-acting sodium channel blocking compound is tetrodotoxin and said effective dose is administered from a formulation containing tetrodotoxin at a concentration between about 0.01 mM to 0.2 mnM.

19. The method of claim 18, wherein said tetrodotoxin is administered in a vehicle having a pH of between about 4-8.

20. A method of reducing pain in a mammal following corneal refractive surgery, comprising,

topically administering to a partially or completely de-epithelialized corneal surface of an eye of said mammal, in an ophthalmically suitable vehicle, a pain reducing effective dose of a pharmaceutical composition consisting essentially of a long-acting sodium channel blocking compound.

21. The method of claim 20, further comprising the step of instilling drops of a non-steroidal anti-inflammatory compound in the eye of said mammal.

22. The method of claim 20, further comprising administering to said mammal an antibiotic or a non-steroidal anti-inflammatory drug.

23. A method of reducing pain in a mammal following corneal refractive surgery, comprising

topically administering to a corneal surface of an eye of said mammal, in an ophthalmically suitable vehicle, a pain reducing effective dose of a pharmaceutical composition consisting essentially of a long-acting sodium channel blocking compound, wherein said administering is by applying to the eye of said mammal a bandage contact lens, wherein said lens is capable of delivering said long-acting sodium channel blocking compound to said corneal surface.

24. As an article of manufacture, an ophthalmically acceptable dosage container comprising:

(a) a pharmaceutical composition consisting essentially of from about 0.003 .mu.g to 160 .mu.g of tetrodotoxin in an ophthalmically acceptable vehicle at pH of between about 4-8.

25. The article of claim 24, wherein said container comprises: from about 0.127 .mu.g to 2.54 .mu.g of tetrodotoxin.

26. A local anesthetic composition which consists essentially of an anesthetically effective amount of tetrodotoxin, in a concentration of 0.01 mM to 1 mM, in an ophthalmically acceptable vehicle at pH 4-6.

27. The composition of claim 26, wherein said concentration is between 0.01 mM and 0.2 mM tetrodotoxin.

28. The composition of claim 26, wherein said concentration is between 0.01 mM and 0.1 mM tetrodotoxin.

29. A local anesthetic composition which consists essentially of an anesthetically effective amount of saxitoxin, in a concentration of 0.1 mM to 10 mM, in an ophthalmically acceptable vehicle at pH 4-8.

30. A local anesthetic composition which consists essentially of an anesthetically effective amount of tetrodotoxin, in a concentration of 0.1 mM to 10 mM, in an ophthalmically acceptable vehicle at pH 4-6.

31. A method of producing a non-toxic local anesthesia in an epithelial tissue region of a mammal, comprising

topically administering an anesthetically effective dose of a pharmaceutical composition consisting essentially of a long-acting sodium channel blocking compound, in a pharmaceutically suitable vehicle comprising a citrate buffer at pH 4-8, to said epithelial tissue region of said mammal.

32. The method of claim 31, wherein said pH is 4-5.

33. The method of claim 31, wherein said anesthesia has a duration of 4-8 hours.

34. The method of claim 31, wherein said long-acting sodium channel blocking compound does not inhibit re-epithelialization of said epithelial tissue.

35. A non-toxic local anesthetic composition which consists essentially of an anesthetically effective amount of tetrodotoxin, in a concentration of 0.01 mM to 10 mM, in an ophtalmically acceptable vehicle comprising citrate buffer at pH 4-8.

36. The composition of claim 35, wherein said pH is 4-5.

37. The composition of claim 35, wherein said pH is 4-5.

38. A non-toxic local anesthetic composition which consists essentially of an anesthetically effective amount of saxitoxin, in a concentration of 0.01 mM to 10 mM, in an ophtalmically acceptable vehicle comprising citrate buffer at pH 4-8.

Description

FIELD OF THE INVENTION

The present invention relates to a method for producing local anesthesia by topical administration of sodium channel blocking compounds, including tetrodotoxin and saxitoxin.

BACKGROUND OF THE INVENTION

Pain is a well known phenomenon as an indicator of actual or potential injury or tissue damage due to inflammation, ischemia, mechanical or other irritation. Treatment of pain includes the use of local anesthetics, which block neuronal transmission and affect sensation as well as pain, and analgesics, which relieve pain and additionally may interfere with the activity of chemical mediators of inflammation.

Loss or damage of epithelial tissue is usually associated with moderate to severe pain and can result from a number of causes, for example, burns, corneal abrasions, other abnormalities of mucosal tissues, and surgical procedures involving epithelial and other tissues.

An example of pain associated with a surgical procedure is surgical correction of myopia by excimer laser photorefractive keratectomy. Following photorefractive keratectomy (PRK), patients generally experience moderate to severe eye pain in the first 24 to 48 hours. Current pain management with bandage contact lens, non-steroidal anti-inflammatory agents, and oral analgesics mitigates, but does not eliminate, the discomfort in most patients (Cherry, Tutton). Topical anesthetics have been used to reduce pain, but due to their short duration of action, frequent administration is required. For example, benoxinate, cocaine, tetracaine, and proparacaine are commonly prescribed topical anesthetics for management of eye pain. These topical anesthetics only provide pain relief for short periods, on the order of 15 to 30 minutes. Given frequently, these agents can be toxic to the corneal epithelium and inhibit re-epithelialization (Rosenwasser).

Thus, there is a need in the art for methods of producing long-lasting, local anesthesia without inhibiting re-epithelialization or healing of other tissues.

SUMMARY OF THE INVENTION

The methods and compositions of the present invention solve, inter alia, the long-recognized need in the art for methods of producing local anesthesia of long duration. In the particular embodiment of producing long-lasting local anesthesia of the corneal surface of an eye, the inventors have addressed a problem of great clinical significance, showing for the first time that sodium channel blocking compounds, such as tetrodotoxin and saxitoxin, can produce ocular surface anesthesia of long duration without impairing re-epithelialization. Moreover, the inventors have shown that the effective doses of those sodium channel blocking compounds have a wide margin of safety and that systemic absorption of tetrodotoxin topically administered to abraded corneas is low.

The methods and compositions of the invention can be used for any condition involving ocular surface pain, including local anesthesia following ocular surgery, including PRK, and following injury to the eye. The methods provide significant advantages, including providing at least 3 hours, preferably at least 4 hours, more preferably at least 6 hours, and most preferably, at least 8 hours of local anesthesia without affecting re-epithelialization of the corneal surface (e.g. wound healing).

The present invention includes methods of producing long-lasting local anesthesia, comprising administering a pharmaceutically acceptable composition of a long-acting sodium channel blocking compound, wherein said compound binds to the extracellular mouth of the sodium channel, occluding the channel by a mechanism separate from that of local anesthetics, such as proparacaine. Preferably, such methods achieve local anesthesia of long duration, lasting at least 3 hours (3 to 10 hours), preferably at least 4 hours (4-10 hours), and most preferably at least 6 to 10 hours. Preferred compounds include toxins or analogs thereof that specifically bind to a site formed in part by an extracellular region of the alpha subunit of a sodium channel. Most preferred compounds comprise the class of toxins and analogs that specifically bind to a site formed by the SS1 and SS2 extracellular regions of the alpha subunit of a sodium channel, wherein such compounds include tetrodotoxin, saxitoxin and analogs thereof. Surprisingly, these long-acting sodium channel blocking compounds, which are well known, potent neurotoxins, provide long-lasting local anesthesia without inhibiting reepithelialization.

Accordingly, it is an object of the invention to provide a method of producing local anesthesia in patients experiencing pain associated with damage to epithelial tissue.

It is another object of the invention to provide a method of producing local anesthesia for long-acting pain control.

It is another object of the invention to provide a method of producing local anesthesia in epithelial tissues having damage associated with corneal abrasions, as in, for example, ophthalmic surgery, such as post-operative photorefractive keratectomy, other forms of corneal refractive surgery, including excimer laser PRK and LASIK, without impairing healing of the epithelial tissue. Other applications include, but are not limited to, any condition where ocular surface anesthesia of long duration is desired, including after surgery or injury.

In one aspect, the invention includes a method of producing local anesthesia in a subject experiencing pain in an epithelial tissue region. The method includes topically administering to the region, in a suitable pharmaceutical vehicle, an effective dose of tetrodotoxin or saxitoxin.

In one embodiment, the effective dose of tetrodotoxin or saxitoxin is administered from a formulation containing tetrodotoxin or saxitoxin at a concentration of between 0.001 mM and 10 mM.

In another embodiment, tetrodotoxin or saxitoxin is administered to a de-epithelialized corneal tissue region. For example, tetrodotoxin is administered to the eye following excimer laser photorefractive keratectomy by instillation of drops. In this application, tetrodotoxin is typically formulated in a vehicle having a pH of between 4-8, more preferably between about 5-7.5.

In one embodiment, tetrodotoxin or saxitoxin is administered topically every 6-8 hours for between about 24-72 hours.

The method of the invention, in another embodiment, is for producing local anesthesia by topical administration of tetrodotoxin to an epithelial tissue region in the upper or lower gastrointestinal tract. In other embodiments, the epithelial tissue region is associated with genital lesions in the genital area, with epithelial tissue region is in the esophagus, or with facial epithelial tissue.

In another aspect, the invention includes a method of producing local anesthesia in the eye of a mammalian subject, by topically administering to the corneal surface of the eye of the subject, in a pharmaceutically suitable vehicle, a pharmaceutically effective dose of tetrodotoxin, saxitoxin or other long-acting sodium channel blocking compound. Such methods find application whenever ocular surface anesthesia is desired.

In one embodiment of this aspect, the corneal surface is partially or completely de-epithelialized.

In a third aspect, the invention includes a method of reducing pain following corneal surgery, including but not limited to excimer laser photorefractive keratectomy, by topically administering to the corneal surface of the eye of the subject, in a pharmaceutically suitable vehicle, a pharmaceutically effective dose of tetrodotoxin or saxitoxin.

In one embodiment of the third aspect, the method also includes the step of instilling drops of a non-steroidal anti-inflammatory compound in the eye of the subject. In other embodiments, the method further includes applying a bandage contact lens to the eye of the subject, or administering an antibiotic, steroid, or non-steroidal anti-inflammatory drug to the subject. Methods of administering combination drops to the eye are also contemplated, wherein such drops contain a composition comprising a long-acting sodium channel blocking compound and an antibiotic, steroid, or nonsteroidal anti-inflammatory drug in an opthalmically acceptable vehicle.

In yet another embodiment, the invention includes methods of producing local anesthesia in an eye of a mammal, comprising (a) topically administering to the corneal surface of the eye of said mammal, in a pharmaceutically suitable vehicle, 0.05%-0.5% proparacaine and (b) topically administering to the corneal surface of the eye of said mammal, in a pharmaceutically suitable vehicle, an opthalmically effective dose of a long-acting sodium channel blocking compound, wherein step (b) follows step (a). In a preferred embodiment, a 0.05% proparacaine concentration is administered.

Also provided are compositions of a long-acting sodium channel blocking compound in an opthalmically acceptable pH range of between 4-8, wherein the concentration of said compound is not greater than 1 mM (0.01 mM to 1 mM), preferably not greater than 0.5 mM (0.01 mM to 0.5 mM), more preferably not greater than 0.2 mM (0.01 mM to 0.2 mM), and most preferably not greater than 0.1 mM (0.01 mM to 0.1 mM).

These and other objects and features of the invention will be more fully appreciated when the following detailed description of the invention is read in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is a plot showing corneal blink response in rabbit eyes as a function of time after administration to the cornea with intact epithelium of 0.1 mM (open circles), 1 mM (open diamonds) and 10 mM (open squares) tetrodotoxin;

FIG. 1B is a plot showing corneal blink response in fellow rabbit eyes for FIG. 1A following topical administration of the vehicle only control;

FIG. 2A is a plot showing corneal blink response in rabbit eyes with intact corneal epithelium as a function of time following topical administration of 1 mM tetrodotoxin, 10 mM tetrodotoxin or a comparative anesthetic, 0.5% proparacaine;

FIG. 2B is a plot showing corneal blink response in fellow rabbit eyes for FIG. 2A following administration of the vehicle only control;

FIGS. 3A-3B are plots showing corneal blink response in rabbit eyes following central corneal epithelial debridement and administration of 0.01 mM (open circles), 0.1 mM (open diamonds) or 1.0 mM tetrodotoxin (open squares) (FIG. 3A) and of the fellow eyes treated with a vehicle only control (FIG. 3B);

FIG. 4 is a plot showing corneal blink response as a function of time in rabbit eyes with central corneal epithelial debridement treated with doses of 1.0 mM tetrodotoxin administered every 6 hours for 24 hours; and

FIG. 5 is a plot showing the size of a corneal epithelial defect, in mm.sup.2, as a function of time for untreated rabbit eyes (open squares) and for rabbit eyes treated with topical 1 mM tetrodotoxin every 8 hours (open diamonds).

FIG. 6 is a chart showing administration of TTX to produce corneal anesthesia of rabbit eyes post-excimer laser keratectomy as measured by a blink response test. Corneal sensation was tested at 3, 6, 9, 12, 15, 18, 21, 24, 30, 32 and 40 hours after excimer laser keratectomy. One 40 microliter drop of 1 mM TTX or vehicle (control) was administered at 0, 6, 12, 18 and 24 hours, as shown by the arrows. The results are graphed as the blink response (mean+/-SEM; N=6).

FIG. 7 is a chart showing the effect of topical administration of TTX on corneal wound healing following excimer laser keratectomy of rabbit eyes. Wound healing was assessed by measuring the size of the epithelial defect remaining at 24, 40, 49, 63, 68 and 72 hours after excimer laser keratectomy. The results are given as the area of the epithelial defect remaining in either the eyes treated with 1 mM TTX or the fellow eyes treated with the vehicle without TTX (control) (mean+/-SD; N=6).

FIG. 8 is a chart showing corneal anesthesia in abraded rabbit eyes following topical application of 20 microliters of 0.1 mM TTX. Corneal sensation was tested for TTX-treated and vehicle treated (control) eyes at 4 and 6 hours after a single topical administration of TTX or the control. The chart shows that a single 20 ul dose of 0.1 mM TTX, when applied to abraded rabbit corneas, resulted in 6 hours of local anesthesia as measured by the blink response.

FIG. 9 is a chart showing corneal anesthesia in abraded rabbit eyes following topical application of 20 microliters of 0.2 mM TTX. Corneal sensation was tested for TTX-treated and vehicle treated (control) eyes at 4, 6 and 8 hours after a single topical administration of TTX or the control. The chart shows that a single 20 ul dose of 0.2 mM TTX, when applied to abraded rabbit corneas, results in 8 hours of local anesthesia as measured by the blink response.

FIG. 10 is a chart showing corneal sensitivity after topical administration of a single dose of either 1 mM STX or vehicle (control) to abraded rabbit corneas as measured by blink response at 2, 4 and 6 hours after administration.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

The following terms, as referred to herein, have the following meanings, unless otherwise indicated.

"Long-acting sodium channel blocking compound" refers to a compound, e.g. a toxin or analog that, when administered to a mammal in an effective concentration, causes local anesthesia lasting at least 3 to 10 hours, and specifically binds to the extracellular mouth of the sodium channel, occluding the channel by a mechanism separate from that of local anesthetics, such as lidocaine, proparacaine. See J. F. Butterworth and G. R. Strichartz, Anesthes. 72:711-734 (1990). Long-acting sodium channel blocking compounds, when administered in a single dose, may effect local anesthesia of long duration, lasting at least 3 hours (3 to 10 hours), preferably at least 4 hours (4-10 hours), and most preferably at least 6 to 10 hours. Such long-acting sodium channel blocking compounds include compounds that specifically bind to a site formed in part by an extracellular region of the alpha subunit of a sodium channel. See Goodman & Gilman's, The Pharmacological Basis of Therapeutics, Ninth Edition 340-341 (1996); H. Terlau, et al., Fed. Europ. Biochem. Soc. 293(1-2): 93-96 (1991); Encyclopedia of Molecular Biology, pages1127-1131 (ed. J. Kendrew 1994). Examples of long-acting sodium channel blocking compounds that bind to an extracellular site formed by the SS1 and SS2 segments of the alpha subunit include but are not limited to tetrodotoxin, saxitoxin, chiriquitoxin, GTTX (from G. tamarensis), gonyautoxins (GTX-I-V, GTX-I, GTX-II, GTX-III), neosaxitoxin, and derivatives and analogs thereof. D. J. Bower, et al., Clinical Toxicology, 18(7):813-863 (1981). Examples of long-acting sodium channel blocking compounds that bind to an extracellular site formed by the SS3 and SS4 segments of the alpha subunit, include but are not limited to alpha-scorpion toxin and sea anemone toxin. See Rogers, J. C. et al., J. Biol. Chem. 271(27):15950-15962 (1996).

"Saxitoxin" or "STX" refers to a compound comprising a tetrahydropurine moiety composed of two guanidine units fused together in a stable azaketal linkage, having a molecular formula C.sub.10 H.sub.27 N.sub.7 O.sub.4.2--HCl, (mol. wt. 299.30) and to derivatives thereof, including but not limited to hydroxysaxitoxins and neosaxitoxin. Bower et al., Nonprotein Neurotoxins, Clin. Toxicol. 18(7):813-863 (1981).

"Tetrodotoxin" or "TTX" refers to the amino perhydroquinazoline compound having the molecular formula C.sub.11 H.sub.17 N.sub.3 O.sub.8 and to derivatives thereof, including but not limited toanhydrotetrodotoxin, tetrodaminotoxin, methoxytetrodotoxin, ethoxytetrodotoxin, deoxytetrodotoxin and tetrodonic acid (Kao). Examples of TTX analogs include novel TTX analogs isolated from other organisms, as well as those that are partially or totally chemically synthesized. See e.g., Yotsu, M. et al. Agric. Biol. Chem., 53(3):893-895 (1989). Such analogs bind to the same site on the alpha subunit of sodium channels as does TTX.

"Anesthesia" refers to the loss of sensation, and, as used herein encompasses analgesia, the reduction in perceived pain without necessarily loss of sensation.

"Topical administration or topically administering" refers to application to a tissue of a mammal, including but not limited to application to epithelial tissue which has been damaged, lost or de-epithelialized.

"Epithelial tissue region" refers to an area of epithelial tissue in a mammal, where the epithelial layer is intact, damaged, or partially or completely absent.

"Pharmaceutically acceptable dose" refers to administration of an amount of a long-acting sodium channel blocking compound effective to achieve a local anesthetic effect for a clinically useful period of time.

"Opthalmically acceptable dose" refers to administration of an amount of a long-acting sodium channel blocking compound effective to achieve a local anesthetic effect in an eye for a clinically useful period of time.

II. Administration of Long-acting Sodium Channel Blocking Compounds

The invention is directed to a method of providing local anesthesia to a mammal experiencing pain in a tissue, preferrably an epithelial tissue region. The method includes topically administering to the region, an effective dose of a long-acting sodium channel blocking compound in a suitable vehicle, including an opthalmically suitable vehicle.

In general embodiments of the invention, described in more detail below, the method provides local anesthesia to a patient having pain in an epithelial tissue region associated with damage or loss of epithelial tissue as a result of, for example, plastic surgery, canker sores, burns, sore throats, genital lesions, upper or lower gastrointestinal bronchoscopy or endoscopy, intubation, dermatologic abrasions or chemical skin peels.

In one preferred embodiment, the method is for producing local anesthesia in de-epithelialized corneal tissue in the eye of a patient after injury to the eye, for example, photorefractive keratectomy, by topically administering to the eye an effective dose of a long-acting sodium channel blocking compound in a suitable vehicle.

In experiments performed in support of the present invention, the use of tetrodotoxin as a topical anesthetic was demonstrated by administration to corneal epithelial tissue in rabbit eyes. Tetrodotoxin was administered to healthy rabbit eyes and to de-epithelialized rabbit eyes to determine the extent and duration of local anesthesia provided by tetrodotoxin and to evaluate its toxicity, as will be described below.

A. Administration in Healthy Rabbit Eyes

1. Administration of 0.1 mM, 1 mM and 10 mM tetrodotoxin

The extent and duration of local anesthesia provided by tetrodotoxin was determined by administration of the anesthetic at concentrations of 0.1 mM, 1 mM and 10 mM to healthy rabbit eyes. As described in Example 1, tetrodotoxin was administered to the right eye of 18 rabbits by instillation of 40 .mu.l of the drug in a sodium citrate vehicle. The fellow, left eye of each rabbit received 40 .mu.l of the sodium citrate vehicle as a control.

The anesthetic effect was determined by the corneal blink test, as described in Example 1. In this test, the cornea of each rabbit was mechanically stimulated and the rabbit's response was scored on a scale of 1-3, where a score of 1 was assigned for no blink, a score of 2 was assigned for a partial blink without full lid closure and a score of 3 was assigned for a full blink. Each eye was scored prior to administration of the drug and at intervals after administration for 8 hours.

FIG. 1A shows the results of administration of tetrodotoxin, where the response score is plotted as a function of time after administration of 0.1 mM (open circles), 1 mM (open diamonds) and 10 mM (open squares) tetrodotoxin. At a concentration of 0.1 mM tetrodotoxin, no local anesthesia was produced in the rabbits, as evidenced by the response scores of 2.8-3.0.

The 1 mM tetrodotoxin formulation produced local anesthesia in all of the animals, as evidenced by the one minute score of 1.17. The local anesthesia effect was relatively short-lived, as evidenced by the 1 hour score of 1.5 and by the 3 hour score of 2.83.

The 10 mM tetrodotoxin formulation produced longer-lasting local anesthesia. At one minute after administration, all rabbit corneas werelocally anesthetic with a mean anesthesia score of 1.0 (SD=0). At 4 hours, local anesthesia was still present with a mean score of 1.17 (SD=0.4 1), and by 8 hours, the mean score of 2.0 indicated residual local anesthesia in most animals. As late as 8 hours, 5 of 6 rabbits showed some residual local anesthesia.

FIG. 1B shows the corneal response scores following administration of the placebo control vehicle. As

seen, corneal blink response remained at a score of about 3, indicating that none of the eyes treated with the control vehicle had diminished corneal sensitivity.

2. Comparative Test

In a separate experiment (Example 1B), the anesthetic duration provided by tetrodotoxin (10 mM and 1 mM) was compared to that provided by 0.5% proparacaine, a common topical ocular anesthetic (Rosenwasser). Following the procedure set forth in Example 1, the right eye of each of the 18 test rabbits received 40 .mu.l of the test drug with the fellow, left eye of each rabbit receiving a placebo control. The results are shown in FIGS. 2A and 2B.

As seen in FIG. 2A, the 10 mM tetrodotoxin dose (open squares) produced significantly longer local anesthesia than proparacaine (open circles). While proparacaine produced local anesthesia in 6 of 6 rabbits at 1 minute (Table 2), by 1 hour the mean blink response score had increased to 2.50 (SD+0.84), and at 3 hours, all eyes receiving proparacaine had normal sensation. In contrast, as late as 5 hours, 4 of 6 rabbits receiving tetrodotoxin showed some residual local anesthesia with a mean score of 1.83, which was significantly different (p=0.0325, Wilcoxon test) from the mean score of 3.00 obtained with proparacaine at 5 hours.

3. Slit Lamp Examination, Pachometry, Toxicity

To assess whether administration of tetrodotoxin causes clinical alterations in the cornea, the eyes of animals treated with 10 mM and 1 mM tetrodotoxin were examined at 12 and 24 hours after drug administration by slit lamp biomicroscopy with a portable Kowa slit lamp, with and without fluorescein stain from impregnated strips moistened with balance salt solution. There was no apparent ocular irritation or epithelial toxicity after administration, nor was there any obvious discomfort, as evidenced by prolonged eye closure or repetitive blinking.

To evaluate whether endothelial function was significantly affected by administration of tetrodotoxin, pachometry (Humphrey Pachometer) readings prior to and 24 hours after tetrodotoxin administration were performed on animals treated with 10 mM and 1 mM tetrodotoxin. Pachometry readings on rabbit eyes receiving the highest doses of tetrodotoxin showed no evidence of corneal thickening during the 24 hour observation period, as shown in Table 3 in Example 1B.

With respect to systemic toxicity, each test rabbit was observed carefully for any signs of systemic toxicity during the 24 hour test period. No rabbit had any alterations of feeding, movement, respiration, or alertness during this period that might suggest a toxic effect of tetrodotoxin. No rabbit died or was noted to have abnormalities in behavior.

Collectively, and in summary, these experiments demonstrate that tetrodotoxin, administered topically by instillation of drops into the eye, provides an anesthetic effect. The dosage administered from the 10 mM tetrodotoxin formulation achieved corneal anesthesia with a rapid onset and anesthetic duration for nearly 4 hours, with some anesthetic effect, as evidenced by reduced corneal sensation, provided for at least 8 hours, a significant improvement over that provided by proparacaine. The tests also showed a dose response effect, with lower doses producing either shorter or no anesthetic effect. Importantly, there was no apparent signs of ocular irritation, corneal thickening or systemic toxicity after administration of tetrodotoxin.

B. Administration in De-epithelialized Rabbit Eyes

In other experiments performed in support of the invention, tetrodotoxin was administered to de-epithelialized rabbit corneas. As set forth in Example 2A, a central epithelial corneal abrasion was created in each eye and tetrodotoxin, at a concentration of 0.1 mM, 1 mM or 10 mM, was administered into the inferior conjunctival cul-de-sac of one eye. A control vehicle was administered into the fellow eye.

Corneal sensation was tested, as described in Example 2A, and the rabbit's response was scored as

described above on a scale of 1-3, with a score of 3 indicating full responsiveness and a score of 1 indicating full local anesthesia. Corneal sensation was tested prior to administration of tetrodotoxin and after administration at 2, 4, 6 and 8 hours (Example 2B).

FIG. 3A shows corneal blink response in centrally de-epithelialized rabbit corneas treated with topical 0.01 mM (open circles), 0.1 mM (open diamonds) or 1.0 mM tetrodotoxin (open squares). FIG. 3B shows the corneal blink response for the fellow, left eyes treated with the vehicle only control.

As seen in FIG. 3A, tetrodotoxin induced local anesthesia of de-epithelialized corneas varied as a function of dose. At 2 hours after tetrodotoxin application, all of the tetrodotoxin concentrations showed some anesthetic effect. At 4 hours, rabbit eyes that were treated with 1.0 mM and 0.1 mM tetrodotoxin were significantly different, with mean anesthesia scores of 1.00 (SDD=0.00, P=0.0011) and 1.67 (SD=0.52, P=0.0011), respectively. At 6 hours after tetrodotoxin administration, 5 of 6 rabbit eyes treated with 1.0 mM tetrodotoxin were anesthetic with a mean response score of 1.50 (SD=0.84, P=0.0076). By 8 hours the mean response score for 1.0 mM tetrodotoxin was 2.50 (SD=0.84), with 2 of 6 rabbits showing significantly reduced sensation.

The rabbits were observed for changes in feeding habits, movement, respiration and alertness for 24 hours, with no apparent changes observed.

The effectiveness of tetrodotoxin produced by repeated dosing was determined, as described in Example 2C. Tetrodotoxin at a concentration of 1 mM was administered every 6 hours to the centrally de-epithelialized cornea of six rabbits for 24 hours. Corneal sensation was measured every 3 hours for 30 hours.

The results, plotted in FIG. 4, show that at 3 hours after the first dose of tetrodotoxin (at t=0) all of the rabbit eyes were anesthetic with a mean response score of 1.00 (SD=0.00, P=0.0011). All six rabbit eyes remained anesthetic for the duration of the experiment, with the 40 .mu.l dose of 1 mM tetrodotoxin administered every six hours as indicated by the arrows in the figure. The mean response scores range from 1.00 to 1.17 (P=0.0011) over the 24 hour period of administration. At 6 hours after the final dose of tetrodotoxin (30 hours after the initial dose), 6 of 6 rabbit eyes were still anesthetic with a mean response score of 1.17 (SD=0.41, P=0.0011). At 10 hours after the final dose (34 hours after the initial dose), 5 of 6 rabbit eyes had normal sensation with a mean response score of 2.83 (SD=0.41).

The results presented in FIGS. 3 and 4 show that tetrodotoxin is an effective anesthetic at a dosage provided by the 1 mM formulation in partially de-epithelialized corneas. The dose required for effective local anesthesia is significantly reduced in de-epithelialized tissues compared to intact corneas, as evidenced by comparing FIG. 1A with FIG. 3. The effective dose administered from the 1 mM. formulation in rabbits is approximately 1% of the lethal human dose, as discussed in more detail below.

The studies also show that tetrodotoxin administered every 6 hours for 24 hours from formulation having a 1 mM concentration of tetrodotoxin produced local anesthesia for greater than 30 hours and that, at this dosing frequency, a reduction in corneal sensation was observed for 30 hours.

C. Re-epithelialization of Tissue

As discussed above, ocular pain after a photorefractive keratectomy procedure is severe for 24-48 hours and characterized by throbbing, watering and foreign body sensations. Conventional treatment includes topical application of non-steroidal anti-inflammatory drugs, low dose topical anesthetics, oral analgesics or bandage soft contact lens. See R. Lim-Bon-Siong, et al., Efficacy and Safety of the ProTek (Vifilcon A) Therapeutic Soft Contact Lens After Photorefractive Keratectomy, Am. J. Opthalmology, 125:169-176 (1998). It has been shown that frequently applied topical anesthetics inhibit corneal re-epithelialization (Rosenwasser).

To determine the effect of tetrodotoxin on corneal re-epithelialization, 1 mM tetrodotoxin was administered to rabbit corneas having an epithelial defect. The epithelial defect was created in the right eye of 12 rabbits using n-heptanol soaked filter paper discs, as described in Example 3. The rabbits were

randomized into two test groups for treatment with 40 .mu.l of 1 mM tetrodotoxin every 8 hours or for no treatment. The size of the defect was measured in both test groups at regular intervals after creation of the defect. The results are shown in FIG. 5.

As seen in FIG. 5, corneal defects of corneas left untreated and of corneas treated with tetrodotoxin were essentially identical in their rates of healing. By 49 hours, 4 of 6 untreated and 3 of 6 tetrodotoxin-treated corneas were completely healed. At 56 hours, 4 of 6 untreated and 4 of 6 tetrodotoxin-treated corneas were completely healed. By 66 hours, all but one cornea from each group had completely healed.

To determine if tetrodotoxin treatment had altered corneal thickness, pachometry was performed 90 hours after the initial wound was created. There was no statistical difference in corneal thickening between untreated and tetrodotoxin-treated corneas.

This experiment shows that topical tetrodotoxin administered every 8 hours had no effect on corneal re-epithelialization compared to untreated control rabbit eyes. The rate of healing was nearly identical for the two groups, and tetrodotoxin had no apparent effect on corneal thickness compared to untreated control eyes.

III. Method of Administration

As discussed above, the present invention provides a method of producing local anesthesia in a subject experiencing pain in an epithelial tissue region, typically an epithelial tissue region that is damaged or is partially or completely absent, by administering a therapeutically effective dose of tetrodotoxin.

Tetrodotoxin is a nonprotein neurotoxin which is found in multiple diverse animal species, including puffer fish, porcupine fish, goby fish, newts, frogs and the blue-ringed octopus (Bower). Tetrodotoxin and its anesthetic properties have been reviewed (Blakenship, Kao, Ogura).

In the experiments described above, tetrodotoxin was administered topically to the cornea of rabbit eyes and found to provide long-acting pain relief with no signs of systemic or local toxicity.

More generally, the method of the present invention is intended for use in providing local anesthesia for pain associated with any epithelial tissue region in a subject, for example, pain associated with epithelial ulcers, such as a canker sore or genital lesions. Canker sores can occur alone or in groups on the inside of the cheek or lip or underneath the tongue. Severely affected people have continuously recurring ulcers which last for one to two weeks (Clayman). Long-acting, topical painkillers, such as tetrodotoxin, can ease the pain without significantly retarding healing of these oral lesions.

Genital ulcers are usually caused by sexually transmitted diseases, including herpes and syphilis. The early stages of syphilis are characterized by a hard chancre, a painful ulcer where bacteria has penetrated the skin. This may be followed by shallow, elongated ulcers once the chancre has healed. Such ulcers are painful. Genital ulceration may also be a side effect of drugs taken orally or caused by solutions applied to genital warts. Tetrodotoxin administered in accordance with the method of the invention provides relief from the pain associated with such ulcers.

Pain in epithelial tissue is also caused by burns. Burns affecting the epidermal layer are usually associated with pain, restlessness and fever. Treatment of such a burn in accordance with the method of the invention can provide relief from the attendant pain, while allowing epithelial healing.

Pain as a result of damage to or loss of epithelial tissue is also associated with other conditions and procedures, such as sore throats and plastic surgery, for example carbon dioxide laser surgery to remove for skin resurfacing and removal of wrinkles (Rosenberg), burns, genital lesions, upper or lower gastrointestinal bronchoscopy or endoscopy, intubation, dermatologic abrasions or chemical skin peels. Tetrodotoxin administered in accordance with the method of the invention is beneficial in relieving pain associated with such damaged tissues without significantly retarding healing.

A. Tetrodotoxin and Saxitoxin Formulations and Dosages

For use in the eye, tetrodotoxin and saxitoxin are typically administered in an aqueous solution, which may be an aqueous suspension, an ointment, a gel or an aqueous polymer solution. Preferably, the solution has a pH of between 4-8, more preferably between about 5-7.5. Such ophthalmic formulations suitable for topical and intraocular administration can be formulated in accordance with techniques known to those of skill in the art.

Typically, the active ingredient tetrodotoxin or saxitoxin is formulated into purified water or a physiological saline solution as a major vehicle. However, it will be appreciated that the ophthalmic formulation can contain other components, including, but not restricted to, buffering means to maintain or adjust pH, such as acetate buffers, citrate buffers, phosphate buffers and borate buffers; viscosity increasing agents such as polyvinyl alcohol, celluloses, such as hydroxypropyl methyl cellulose and carbomer; preservatives, such as benzalkonium chloride, chlorobutanol, phenylmercuric acetate and phenyl mercuric nitrate; tonicity adjustors, such as sodium chloride, mannitol and glycerine; and penetration enhancers, such as glycols, oleic acid, alkyl amines and the like. The addition of a vasoconstrictor to the formulation is also contemplated. Combination formulations comprising said long-acting sodium channel blocking compound and an antibiotic, a steroidal or a non-steroidal anti-inflammatory drug are also contemplated.

The ophthalmic formulation may also be a suspension of particles, such as polymer particles or liposomes, for entrapping the active compound. For example, polymer particles prepared by suspension or emulsion polymerization to have a particle size of below about 50 .mu.m, for entrapping the active compound or for increasing viscosity can be added to the formulation.

The final ophthalmic formulations in unit dosage form are preferably stored in opaque or brown containers to protect then from light exposure. Multi-dose containers can also be used, if desired, particularly if the final formulation has a low viscosity to permit constant, accurate dosages to be administered dropwise to the eye.

The method of the invention, more generally, provides local anesthesia to mucous membranes or damaged, e.g., abraded, skin by topical administration of tetrodotoxin or saxitoxin in the form of solutions, creams, ointments, gels, aerosols or the like. Such formulations are prepared using ingredients and according to procedures known to those of skill in the art or as described in, for example, REMINGTON'S PHARMACEUTICAL SCIENCES.

Administration of tetrodotoxin has been well studied in a number of animal species (Kao), and the lethal oral dose in humans has been estimated to be about 10-18 .mu.g/kg (Kao). For a 70 kg person, the lethal dose would therefore be 0.7-1.26 mg.

In the experiments performed in support of the present invention, described above, a 40 .mu.l aliquot of 0.01 mM, 1 mM or 10 mM tetrodotoxin was administered topically to rabbits' eyes. This corresponds to a dosage of between 0.127-127 .mu.g of tetrodotoxin, well below the lethal oral human dose and giving a sufficient safety margin to allow for any differences in systemic absorption between topical and oral administration. In additional experiments, 20 .mu.l aliquots of 0.1 mM and 0.2 mM tetrodotoxin were administered topically to partially de-epithelialized rabbit eyes, resulting in 6 and 8 hours of local anesthesia respectively.

For administration to the eye, the tetrodotoxin formulation, as a concentration of between 0.001-10 mM and in the form of aqueous solution, suspension, ointment or the like, is administered dropwise to the eye. A single drop typically administers between 10-50 .mu.l. The drop volume and solution concentration, of course, determine the dosage delivered, which, for the ranges specified here, is between about 0.003-160 .mu.g.

Saxitoxin (1 mM) was administered in a 20 .mu.l dose to partially de-epithelialized rabbit corneas, resulting in 4 hours of local anesthesia. This dosage of saxitoxin corresponds to approximately 5.9 .mu.g of saxitoxin, well below the estimated human lethal dose (oral) of between 300 .mu.g to 1.0 mg. See

Bower et al., Clin. Toxicol., 18(7):813-863 (1981).

For administration to the eye, the saxitoxin formulation, as a concentration of between 0.001-10 mM and in the form of aqueous solution, suspension, ointment or the like, is administered dropwise to the eye. A single drop typically administers between 10-50 .mu.l. The drop volume and solution concentration, of course, determine the dosage delivered, which, for the ranges specified here, is between about 0.003 to 149 .mu.g.

It will be appreciated that the dose and concentration of tetrodotoxin or saxitoxin administered is determined on an individual basis, with consideration given to such factors as age and body weight of the patient, as well as to the route of administration and the clinical anaesthetic requirements.

Preferably, tetrodotoxin or saxitoxin is administered topically to the painful epithelial tissue region by application of a formulation having a tetrodotoxin or saxitoxin concentration of between about 0.001-10 mM. The actual dosage of tetrodotoxin or saxitoxin administered will, of course, depend on the amount of formulation applied and the surface area over which it is applied.

The dosing regimen is selected to provide pain relief as needed for a particular clinical condition. Often, pain is most intense in the first 24-72 hours following damage to the tissue, by a surgical procedure or other trauma, and administration every 6 hours during this time period is effective to provide a long-acting anesthetic effect. This was demonstrated in the experiment discussed above where tetrodotoxin was administered every 6 hours for 24 hours to achieve greater than 30 hours of local anesthesia in rabbits, with no signs of irritation or toxicity.

It will be appreciated that topical administration of either tetrodotoxin or saxitoxin may be combined with conventional modes of pain relief, such as administration of oral or topical non-steroidal anti-inflammatory drugs or antibiotics and the use of bandage soft contact lens in ocular applications. For example, methods of the invention include applying to the corneal surface of an eye of a mammal, a bandage contact lens, wherein said lens is capable of delivering an opthalmically effective dose of said long-acting sodium channel blocking compound to said corneal surface. One of skill in the art will appreciate that the there are a number of ocular drug delivery systems and methods that can be used with the present invention, some of which are described in Lee, V. and Robinson, J. R., Review: Topical Ocular Drug Delivery: Recent Developments and Future Challenges, J. Ocular Pharmacol. 2(1):67-108 (1986) (incorporated herein by reference).

Moreover, another preferred method of producing anesthesia in an eye of a mammal comprises topically administering to the corneal surface of an eye of a mammal an opthalmically effective dose of proparacaine or other comparable local anesthetic, including tetracaine or benoxinate, in an opthalmically acceptable vehicle before administering to said eye, an opthalmically effective dose of a long-acting sodium channel blocking compound in an opthalmically acceptable vehicle. Prior administration of proparacaine or other comparable local anesthetic, to the eye of a mammal effectively diminishes the production of tears by the eye, preparing the eye for topical administration of a long-acting sodium channel blocking compound, such as TTX or STX. By decreasing tearing and anesthetizing the corneal surface of an eye, the prior administration of proparacaine, tetracaine or benoxinate, decreases the amount of the long-acting sodium channel blocking compound that would wash away with tears. Thus, the method enables administration of lower effective doses of the long-acting sodium channel blocking compound. For example, dosages of proparacaine range from 0.05% to 0.5%, and preferably, from 0.05 to 0.1% formulations in any opthalmically acceptable vehicle.

From the foregoing, it can be appreciated how various features and objects of the invention are met. The method of the invention provides an effective, long-lasting local anesthesia by topical administration of a long-acting sodium channel blocking compound to a painful epithelial tissue region. The studies reported herein illustrate, using a rabbit model, that a single dose of tetrodotoxin at a concentration of 1 mM or 10 mM achieves local anesthesia with rapid onset and for at least 8 hours. A repeated dosing regimen is effective to produce local anesthesia for greater than 30 hours. Importantly, no evidence of either ocular or systemic toxicity in the test animals was observed at any of the dosage levels, including 10 mM tetrodotoxin. Moreover, a single dose of saxitoxin at a concentration of 1 mM achieves local

anesthesia upon administration to a partially abraded cornea with rapid onset and for at least 4 hours.

One of skill in the art will appreciate that any of the long-acting sodium channel blocking compounds can be used according to the methods and procedures described herein to determine pharmaceutically or opthalmically effective doses and other aspects of the invention.

VI. EXAMPLES

The following examples illustrate the methods and compositions of the invention, but are in no way intended to limit the invention.

Materials: Tetrodotoxin was purchased from Sigma Chemical Co. (St. Louis, Mo.) in vials containing 1 mg tetrodotoxin and approximately 5 mg sodium citrate buffer, pH=4.3, in lyophilized form.

Saxitoxin and additional tetrodotoxin was purchased from Alexis Chemicals.

Example 1

Administration of Tetrodotoxin to Healthy Rabbit Eyes

A. 0.1 mM, 1 mM and 10 mM Tetrodotoxin

Tetrodotoxin formulations of 0.1 mM, 1 mM and 10 mM were prepared in a 60 mM sodium citrate carrier at pH 4.3.

Eighteen New Zealand white rabbits were divided into three groups of six rabbits. Each rabbit received a 40 .mu.l aliquot of one of the tetrodotoxin formulations into the inferior conjunctival cul-de-sac of the right eye, with the left fellow eye receiving 40 .mu.l of the pH 4.3 60 mM sodium citrate carrier as a control.

Corneal sensation was tested with a 4-0 silk suture mounted upon a wooden cotton tip applicator such that the suture extended 5 mm beyond the wooden end of the applicator. The cornea was mechanically stimulated centrally three times with the suture to produce grossly visible indentation of the cornea as the endpoint (similar to previous rabbit model of corneal anesthesia reported in Maurice D M, Singh T., The absence of corneal toxicity with low-level topical anesthesia. Am J Ophthamol. 99:691-696. (1985)). Care was taken not to stimulate the eyelashes. The rabbit's response was graded in the following fashion: no blink=1; partial blink without full eyelid closure=2; fuill blink=3. Thus, a score of 3 indicates full responsiveness and a score of 1 indicates full local anesthesia. The highest anesthesia score of the 3 tests was recorded for each time point.

Corneal sensation was tested prior to administration of drugs and again at 1 minute, 1 hour, 4 hours and 8 hours. The results are tabulated in Table 1 below and shown in FIGS. 1A-1B. Statistical analysis was by the non-parametric Wilcoxon test with a statistical sugnificance of p-<0.05.

TABLE 1

Drug Treatment	Mean Blink Response (N = 6) 0 min.	1 min.	60 min.	240 min.	480 min.
0.1 mM tetrodotoxin	2.83 (0.41)	2.67 (0.52)	2.67 (0.82)	3.00 (0.00)	

					2.83 (0.41)
0.1 mM tetrodotoxin/control		3.00 (0.00)1)	3.00 (0.00)	3.00 (0.00)	3.00 (0.00)
1 mM tetrodotoxin	2.83 (0.41)	1.17 (0.41)	1.50 (0.84)	2.83 (0.41)	2.83 (0.41)
1 mM tetrodotoxin/control		2.83 (0.41)41)	2.83 (0.41)	3.00 (0.00)	2.67 (0.52)
10 mM tetrodotoxin	3.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.17 (0.41)	2.00 (0.89)
10 mM tetrodotoxin/control		3.00 (0.00)00)	2.83 (0.41)	3.00 (0.00)	3.00 (0.00)

*numbers in parenthesis are standard deviation of mean blink response for n = 6.

B. Comparative Study: 1 mM, 10 mM Tetrodotoxin and Proparacaine

Eighteen New Zealand white rabbits were divided into three groups of six rabbits. Each rabbit received a 40 .mu.l aliquot into the inferior conjunctival cul-de-sac of the right eye of 1 mM or 10 mM tetrodotoxin or of proparacaine 0.5% (Ophthetic). The left fellow eye received 40 .mu.l of a placebo, control vehicle (60 mM, pH 4.3 sodium citrate).

Following the procedure described in Example 1A, the anesthetic duration was determined by measuring corneal blink response in the test rabbits at 0 min, 1 minute, 1 hour, 3 hours and 5 hours. The results are tabulated in Table 2 and shown in FIGS. 2A-2B.

TABLE 2

	Mean BIink Response (N = 6)				
Drug Treatment	0 min.	1 min.	60 min.	180 min.	300 min.
Proparacaine	3.00 (0.00)	1.00 (0.00)	2.50 (0.84)	3.00 (0.00)	3.00 (0.00)
Proparacaine/control	3.00 (0.00)	3.00 (0.00)	2.50 (0.84)	3.00 (0.00)	3.00 (0.00)

```
1 mM tetrodotoxin
          3.00 (0.00)
              2.17 (0.75)
                     2.00 (0.89)
                           2.50 (0.55)
                                  2.83 (0:41)
1 mM          3.00 (0.00)0.00)
                     2.83 (0.41)
                           3.00 (0.00)
                                  3.00 (0.00)
tetrodotoxin/control
10 mM tetrodotoxin
           3.00 (0.00)
              1.00 (0.00)
                     1.00 (0.00)
                           1.33 (0.82)
                                  1.83 (0.98)
10 mM         3.00 (0.00).00)
                     3.00 (0.00)
                           3.00 (0.00)
                                  3.00 (0.00)
tetrodotoxin/control
```

*numbers in parenthesis are standard deviation of mean blink response for n = 6.

The extent and duration of local anesthesia after topical TTX was administered to intact rabbit corneas varied as a function of dose (FIG. 1A, Table 1). At 0.1 mM TTX, only partial local anesthesia was produced in 2 of 6 rabbits. At 1 mM TTX, local anesthesia initially was produced in 6 of 6 animals but the effect was generally short-lived. At one minute after administration of TTX, the mean anesthesia score was 1.17 (SD=0.41). At 1 hour the score was 1.50 (SD=0.84) and by 3 hours the mean score had increased to 2.83 (SD=0.41). At 6 hours the TTX-vehicle had a better anesthetic score than the 1 mM TTX treated eyes. However, neither of these scores were significantly different from each other or from the pretreated eyes anesthesia score of 3.0. (SD=0).

At 10 mM, TTX produced a more reproducible and longer lasting local anesthesia. At one minute after administration, all rabbit corneas were anesthetic with a mean anesthesia score of 1.00 (SD=0). At 4 hours, local anesthesia was still present with a mean score of 1.17 (SD=0.41). As late as 8 hours, 4 of 6 rabbits showed some residual local anesthesia with a mean score of 2.00 (SD=0.89). This was significantly different, (P=0.0325), from the mean score of 3.00 (SD=0) obtained with vehicle alone at 8 hours (Table 1).

The anesthetic duration of 10 mM TTX was compared to that of 1 mM TTX, and proparacaine (FIG. 2, Table 2). At the 10 mM dose, TTX produced significantly longer local anesthesia than proparacaine. While proparacaine produced local anesthesia in 6 of 6 rabbits at 1 minute, by 1 hour the mean score had increased to 2.50 (SD=0.84), and at 3 hours, all eyes receiving proparacaine had normal sensation. As late as 5 hours, 4 of 6 rabbits receiving TTX showed some residual local anesthesia with a mean score of 1.83 (SD=0.98). This was significantly different, (P=0.0325), from the mean score of 3.00 (SD=0) obtained with proparacaine or vehicle alone at 5 hours (Table 2).

C. Pachometry

To evaluate whether endothelial function was significantly affected by administration of 10 mM or 1 mM TTX, pachometry readings were made (Humphrey Pachometer, Humphrey Instr., San Leandro, Calif.) prior to and 24 hours after TTX administration. The results are given in Table 3 as mean corneal thickness, with the standard deviation indicated in parenthesis. Pachometry readings on rabbits receiving the highest doses of TTX showed no evidence of corneal thickening during the 24 hour observation period.

TABLE 3

Drug Treatment	Corneal Thickness (mm) 0 hrs.	24 hrs.
proparacaine	0.38 (0.03)	0.38 (0.04)
proparacame/control	0.39 (0.04)	0.38 (0.03)
1 mM tetrodotoxin		0.39 (0.04)
1 mM tetrodotoxin/control	0.36 (0.01)	0.39 (0.04)
10 mM tetrodotoxin		0.37 (0.01)
1 mM tetrodotoxin/control	0.36 (0.01)	0.38 (0.04)

*numbers in parenthesis are standard deviation of mean corneal thickness for n = 6.

Drug treatments labelled "/control" represent fellow eyes of either proparacaine or TTX-treated eyes, wherein the fellow eyes were treated with the citrate vehicle alone.

Slit Lamp Examination and Fluorescein Staining.

Slit lamp biomicroscopy with a portable slit lamp (Kowa SL-5, Kowa Company, Japan) was performed with and without fluorescein stain from impregnated strips moistened with balance salt solution at 12 and 24 hours after topical administration.

To assess whether TTX administration caused clinical alterations in the cornea, all animals were examined with a slit lamp after fluorescein staining. Despite the acidic vehicle, TTX administration did not cause any apparent ocular irritation after administration. There was no obvious discomfort in any of the rabbits evidenced by prolonged eye closure or repetitive blinking. No ocular injection was noted during the 24 hour observation period. At 3 hours most of the rabbits had a mild central punctuate epithelial keratopathy in the area of corneal sensation testing, but there was no difference between rabbits receiving proparacaine and TTX. By 24 hours, all signs of epithelial damage had disappeared by slit lamp examination and fluorescein staining.

Toxicity

The rabbits were observed for changes in feeding habits, movement, respiration and alertness during the first 24 hours by the experimenters and for the subsequent week daily by animal care personnel.

No rabbit had any alterations of feeding, movement, respiration, or alertness during this period that suggested a toxic effect of the TTX. No rabbit died or was noted to have abnormalities in behavior by the animal care personnel for 7 days subsequent to TTX administration.

Example 2

Topical Administration of Tetrodotoxin to Partially De-epithelialized Rabbit Corneas

A. Corneal Abradement and Blink Response Test

After general anesthesia and topical application of 0.5% proparacaine to each eye, a #69 Beaver blade was used to create a central epithelial defect which measured between 3.0-3.5 mm diameter in both eyes of each test rabbit.

Corneal sensation was tested with a 4-0 silk suture mounted upon a wooden cotton tip applicator such that the suture extended 5 mm beyond the wooden end of the applicator. Because the central cornea was often rendered largely anesthetic following mechanical epithelial debridement, the cornea was stimulated in the mid-peripheral cornea, outside of the abraded area, with the suture to produce grossly visible indentation of the cornea as the endpoint. Care was taken not to stimulate the eyelashes. The rabbit's response was graded in the following fashion; no blink=1, partial blink without full eyelid closure=2, full blink=3.

B. Dose Response

Tetrodotoxin in a pH 4.3 sodium citrate vehicle was formulated into concentrations of 1 mM, 0.1 mM, and 0.01 mM.

New Zealand white rabbits were divided into three experimental groups of six. Each rabbit received a 40 .mu.l aliquot of tetrodotoxin at a concentration of 0.01 mM, 0.1 mM or 1 mM into the inferior conjunctival cul-de-sac of the right eye. A 40 .mu.l of a pH 4.3 sodium citrate vehicle as a control into the fellow, left eye.

Corneal sensation was tested prior to administration of tetrodotoxin and after administration at 2, 4, 6 and 8 hours. The results, presented as the mean score for the 6 rabbits in each test group, are tabulated below in Table 4 and shown in FIGS. 3A-3B, where FIG. 3A shows the response scores for eyes treated with tetrodotoxin and FIG. 3B shows the response scores for the fellow, control-vehicle treated eyes. Statistical analysis using the Wilcoxon test was performed by comparing the tetrodotoxin treated eyes to the pre-operative anesthesia response score.

TABLE 4

Drug Treatment	Mean Blink Response (N = 6) 2 hrs.	4 hrs.	6 hrs.	8 hrs
1.0 mM tetrodotoxin	1.00 (0.00)	1.00 (0.00)	1.50 (0.84)	2.50 (0.84)
1.0 mM tetrodotoxin/control	2.83 (0.41)	3.00 (0.00)	3.00 (0.00)	3.00 (0.00)
0.1 mM tetrodotoxin	1.17 (0.41)	1.67 (0.52)	2.83 (0.41)	3.00 (0.00)
0.1 mM tetrodotoxin/control	2.83 (0.41)	3.00 (0.00)	3.00 (0.00)	3.00 (0.00)
0.01 mM tetrodotoxin	1.50 (0.84)	2.33 (0.82)	2.83 (0.41)	2.67 (0.52)
0.01 mM tetrodotoxin/	2.50 (0.84)	2.67 (0.52)	3.00 (0.00)	3.00 (0.00)

control

*numbers in parenthesis are standard deviation of mean blink response for n = 6.

TTX-induced local anesthesia of de-epithelialized corneas varied as a function of dose. At 2 h after TTX application, all of the TTX concentrations showed some anesthetic effect. TTX at 1.0 mM, 0.1 mM, and 0.01 mM had mean anesthesia scores of 1.00 (SD=0.00, P=0.0011), 1.17 (SD=0.41, P=0.0076), and 1.50 (SD=0.84, P=0.0076) respectively. At 4 h, rabbit eyes that were treated with 1.0 mM and 0.1 mM TTX were still significantly anesthetic with mean anesthesia scores of 1.00 (SD=0.00, P=0.0011) and 1.67 (SD=0.52, P=0.0011), respectively. In contrast, the mean anesthesia score of rabbits treated with 0.01 mM TTX was returning to normal at 2.33 (SD=0.82) by 4 h. By 6 h, rabbit eyes treated with either 0.1 mM or 0.01 mM had mean scores of 2.83 (SD=0.41). At 6 h after TTX administration, five of six rabbit eyes treated with 1.0 mM TTX were still partially anesthetic with a mean anesthesia score of 1.50 (SD=0.84, P=0.0076). By 8 h the mean anesthesia score for 1.0 mM TTX was approaching normal at 2.50 (SD=0.84), with only two of six rabbits showing any anesthetic effect.

C. Dosing Frequency

Experiments were conducted to test whether TIX could produce prolonged effectiveness with repeated dosing. A 40 .mu.l aliquot of 1 mM tetrodotoxin was administered every 6 hours for 24 hours to the centrally de-epithelialized cornea of 6 rabbits. Corneal sensation was monitored, by scoring the eye for blink response as described above, every 3 hours for 24 hours and at 17 hours, 30 hours and 34 hours after the initial dose. Response scores after administration of tetrodotoxin were compared to pre-operative response scores for statiscal analysis by the Wilcoxon test. The results are tabulated in Table 5 and shown in FIG. 4, where the arrows in the figure indicate the dosage times.

TABLE 5

Time (hours)	Blink Response
	3.00 (0.00)
3	1.00 (0.00)
6	1.17 (0.41)
9	1.00 (0.00)
12	1.00 (0.00)
15	1.00 (0.00)
18	1.00 (0.00)
21	1.17 (0.41)
24	1.00 (0.00)
17	1.00 (0.00)
30	1.17 (0.41)
34	2.83 (0.41)

*numbers in parenthesis are standard deviation of mean blink response for n = 6.

At 3 hours after the first administration, all of the rabbit eyes were anesthetic with a mean anesthesia score of 1.00 (SD=0.00, P=0.0011). All six rabbit eyes remained anesthetic for the duration of the experiment, with mean anesthesia scores ranging from 1.00 to 1.17 (P=0.0011 throughout). At 6 h after the final TTX administration and 30 h after the initial TTX administration, six of six rabbit eyes were still anesthetic with a mean anesthesia score of 1.17 (SD=0.41, P=0.0011). At 10 h after the final TTX administration and 34 h after the initial TTX administration, five of six rabbit eyes had normal sensation with a mean anesthesia score of 2.83 (SD=0.41).

Example 3

Administration of Tetrodotoxin to Partially De-epithelialized Rabbit Corneas

A. Corneal Abradement

To determine whether TTX inhibits corneal re-epithelialization, twelve New Zealand White rabbits were anesthetized with a mixture of keamine and xylazine. A corneal trephine was used to make Whatman #2 filter paper discs measuring 7.5 mm in diameter. The discs were soaked in n-heptanol solution and blotted to remove excess liquid. One disc was placed on the central cornea of the right eye of each rabbit for 30 seconds. After removal, the cornea was washed with balanced salt solution to remove the loosened epithelial cells. Topical fluorescein was applied and the diameter of the epithelial defect was measured in 2 meridians (12:00-6:00 and 3:00-9:00) using calipers. The radius of the defect was determined, and the defect area was calculated as previously described using an equation that corrects for the curvature of the rabbit cornea. Crosson C E, Klyce S D, Beuerman R W (1986) Epithelial wound closure in the rabbit cornea. Invest Ophthalmol Vis Sci 27:464-473.

B. Effect of Multiple Doses of TTX on Re-epithelialization of Abraded Rabbit Corneas

The rabbits were randomized into two test groups for treatment with 40 .mu.l of 1 mM tetrodotoxin every 8 hours or for no treatment. The rabbits were re-anesthetized with keratin and xylazine and epithelial defect size was re-measured with calipers at 17, 32, 42, 49, 56, and 66 h after the creation of epithelial defects. Twenty-four hours after the corneal epithelial defects were closed (completely healed), pachometry (Humphrey Ultrasonic Pachometer) was performed in both the experimental and fellow eyes of each rabbit.

The results are shown in FIG. 5 and Table 6.

TABLE 6

Corneal Defect Size

TIME (h)	AREA OF DEFECT (mm.sup.2) CONTROL	TTX	
0		53.0 +/- 2.0	
		49.0 +/- 5.8	
17			37.9 +/- 7.1
32			15.7 +/- 7.1
42			5.6 +/- 6.3
49			3.0 +/- 5.7
56			2.5 +/- 5.9
66			0.3 +/- 0.7

Corneal defects of untreated and 1.0 mM TTX-treated corneas were essentially identical in their healing rates. By 48 h, four of six untreated corneas and three of six TTX-treated corneas were completely healed. At 56 h, four of six untreated and four of six TTX-treated corneas were completely healed. By 66 h, all but one cornea from each group had completely healed. In these remaining eyes, only a small defect, 0.67+/-1.63 mm2 for the TTX-treated rabbit and 0.29+/-0.72 mm2 for the untreated control rabbit, remained

C. Effect of Multiple Doses of TTX on Corneal Thickness after Re-epithelialization

In order to determine whether TTX had an effect on corneal thickness after re-epithelialization compared to untreated control eyes, corneal thickness was measured 92 hours after creation of a corneal abrasion (OD) in rabbit eyes that had been treated with multiple doses of 1.0 mM TTX. Corneal thickness of healed corneal abrasions (OD) and uninjured fellow eyes (OS) were measured by pachometry. The results are given as the mean defect area and the standard deviation (n=6)

TABLE 7

DRUG TREATMENT	CORNEAL THICKNESS OD	OS
NONE	0.39 +/- 0.04	0.43 +/- 0.04
1.0 mM TTX	0.40 +/- 0.04	0.41 +/- 0.05

There was no statistically significant difference in corneal thickness between untreated and TTX-treated corneas (Table 7).

Example 4

Topical Administration of Tetrodotoxin to Rabbit Eyes after Excimer Laser Keratectomy

TTX in a pH 4.3 sodium citrate vehicle was used in the following experiments (Sigma Chemical Co., St. Louis, Mo.). New Zealand white rabbits were divided into two experimental groups, each consisting of six rabbits. After general anesthesia by intramuscular injection of a mixture of xylazine and keratin, followed by topical application of 0.5% proparacaine (Ophthetic, Allergan, Irvine, Calif.) to each right eye, the rabbits underwent excimer laser keratectomy on their right eyes.

Excimer laser keratectomy was performed using the Star Excimer Laser System (VISX, Inc.) in phototherapeutic keratectomy (PTK) mode. Excimer laser keratectomy was performed to create a 5 mm diameter wound, 75 .mu.m in depth. The repetition rate of the laser was set at 6 Hz with a pulse energy density of 160 mJ/cm2.

One group of six rabbits then received a 40 .mu.l aliquot of 1 mM TTX into the inferior conjunctival cul-de-sac of their right eyes and the other group of six rabbits received 40 .mu.l of the pH 4.3 sodium citrate vehicle into the inferior conjunctival cul-de-sac of their right eyes as a control. The rabbits were treated with 1 mM TTX or vehicle again at 6, 12, 18, and 24 hours.

Corneal sensation was tested as previously described. Briefly, sensation was tested with a 5 mm silk suture mounted on a wooden cotton tip applicator. The cornea was stimulated with the suture in the mid-peripheral cornea, outside of the excimer laser keratectomy treated area. Care was taken not to stimulate the eyelashes. The rabbit's response was graded in the following fashion: no blink=1, partial blink without full eyelid closure=2, full blink=3. Corneal sensation was tested at 3, 6, 9, 12, 15 ,18, 21, 24, 30, 32, and 40 hours. At 6, 12, 18 and 24 hours the corneal sensation was tested prior to re-administration of TTX or vehicle. The rabbits were observed for changes in feeding habits, movement, respiration and alertness. The data are presented in FIG. 6 as the mean score of 6 rabbits/treatment group. TTX treated eyes' anesthesia scores were compared to vehicle treated eyes' anesthesia scores for statistical significance by the Wilcoxon test.

Administration of 40 .mu.l of 1 mM TTX every 6 hours for 24 hours after excimer laser keratectomy produced nearly complete local anesthesia for at least 30 hours with a mean anesthesia score of 1.17 (SD=0.41) (p=0.011, Wilcoxon test) (FIG. 6). Three hours following each application of TTX, the mean anesthesia score for TTX treated eyes was 1.0 (SD=0). Six hours following each application of TTX the mean anesthesia scores were between 1.0 (SD=0) and 1.5 (SD=0.84). At least 4/6 of the rabbits were completely anesthetic at six hours following each application of TTX. At 32 hours, 8 hours following the fmal application of TTX, there was still significant local anesthesia of the TTX treated corneas (p=0.0325, Wilcoxon test). The mean anesthesia score was 2.0 (SD=0.89). At 40 hours, 18 hours following the final application of TTX, all of the rabbits' corneas had returned to normal sensation. In contrast, after 9 hours, vehicle treated eyes all had normal sensation for the duration of the experiment (FIG. 6). There was a very slight local anesthesia of the vehicle treated eyes at 6 and 9 hours following excimer laser keratectomy, with the mean anesthesia score being 2.83 (SD=0.41). However, this was due in each case to 1/6 rabbits scoring 2, 5/6 rabbits scoring 3, and was not significant. At 3 hours following excimer laser keratectomy the vehicle treated eye's mean anesthesia score was 2.16 (SD=0.75). This was probably due to lingering effects of the general and topical anesthetics that were

administered prior to the excimer laser keratectomy procedure.

Example 5

Effect of Topical Administration of TTX Following PRK on Corneal Re-epithelialization

To determine whether TTX inhibited corneal re-epithelialization following excimer laser keratectomy, topical fluorescein was applied after rabbits were given general anesthesia by intramuscular injection of a mixture of xylazine and keratin. The diameter of the circular epithelial defect was measured using calipers in 2 meridians (12:00-6:00 and 3:00-9:00), and the radius of the defect was calculated. The radius was used to calculate the area of the defect using the method of Crosson et al. that corrects for the curvature of the rabbit cornea (Crosson C E, et al., Invest Ophthalmol Vis Sci., 27:464-473 (1986)). General anesthesia was administered and the size of the epithelial defect was measured at 24, 40, 49, 63, 68, and 72 hours following excimer laser keratectomy. Observers were masked as to the contents of eye drops given to the rabbits.

To assess whether or not repeated administration of TTX had any effect on the rate of epithelial healing, epithelial defect area was measured over 72 hours following excimer laser keratectomy. As shown in FIG. 7, there was little difference in healing rate of vehicle and TTX treated eyes. At 24 hours there was no significant difference in healing between TTX treated and vehicle treated eyes ($p>0.05$, t-test). At 40 hours the TTX treated eyes had larger defects than vehicle treated eyes 7.85 mm2 vs. 4.54 mm2 ($p<0.025$, t-test). However, at 49 hours, and thereafter, both groups were equally healed ($p>0.05$, t-test).

Toxicity.

The rabbits involved in the corneal anesthesia and corneal wound healing experiments following excimer laser keratectomy described above were observed carefully for any signs of systemic toxicity during the course of the experiment. No rabbit had any alterations of feeding, movement, respiration, or alertness during this period that suggested a toxic effect of the TTX. No rabbit died or was noted to have abnormalities in behavior subsequent to TTX administration. Additionally, no signs of local toxicity such as ocular injection or corneal haze were apparent in any rabbit.

Example 6

Systemic Absorption of TTX Topically Applied to Abraded Rabbit Eyes

TTX (Alexis Corporation) was formulated into 20 .mu.l doses containing 100 .mu.g of m in a pH 4.3 citrate buffer (60 mM). Four Dutch banded rabbits were weighed. After general anesthesia by intramuscular injection of a mixture of xylazine and keratin, followed by topical application of 0.5% proparacaine to each eye, a #69 Beaver blade was used to create a central epithelial defect which measured between 4 mm diameter in one eye of each rabbit. Each rabbit then received a 20 .mu.l aliquot of TTX into the inferior conjunctival cul-de-sac of the abraded eye. At 10 min, 20 min and 40 min following administration of TTX, 4 ml of blood was taken from each rabbit. The blood was clotted and the serum collected.

TTX was purified from serum samples using the purification scheme of Yasumoto and Michishita. Fluorometric determination of tetrodotoxin by high performance liquid chromatography. Agric Biol Chem 49: 3077-3080 (1985). Briefly, 300 .mu.l of each serum sample was extracted by boiling for 10 min in 0.02N acetic acid, and TTX was purified by chromatography over Amberlite CG-50. TTX was eluted from the Amberlite with 0.5 N acetic acid, samples were dried in a vacuum centrifuge and TTX was resuspended in 50 .mu.l of phosphate buffered saline.

Assays to quantify the amount of TTX purified from serum samples were performed using a tissue culture bioassay. Hamasaki, K, Kogure, K and Ohwada, K. A biological method for the quantitative measurement of tetrodotoxin (TTX): tissue culture bioassay in combination with a water-soluble tetrazolium salt. Toxicon 34: 490-495 (1996). Briefly, 2 to 3.times.105 mouse Neuro 2A cells per well were plated on 96 well microtiter plates in 200 .mu.l of RPMI-1640 medium containing 10 fetal bovine

serum and incubated overnight in a 37.degree. C. tissue culture incubator. After 24 hours the cells were given 10 .mu.l of the TTX samples, 20 .mu.l of 10 mM ouabain, and 20 .mu.l of 0.5 mM veratridine. After an additional 24 hours at 37.degree. C., the cells were given 20 .mu.l of WST-1 cell counting reagent (Dojindo Corp.) and allowed to incubate an additional 3 hours at 37.degree. C. The yellow color produced by the WST-1 reagent was quantified by reading the absorbance at 430 nm with a reference wave length of 600 nm. All assays were performed in duplicate. TTX concentrations were derived by comparison to a standard curve generated using normal rabbit serum that had been spiked with known amounts of pure TTX and subjected to the same purification scheme as the rabbit serum samples. The results are given in Table 8 as the average amount of TTX in .mu.g per ml of serum at each time point in the table +/- standard deviation (N=4) (SE=Standard Error). The average amount of TTX absorbed into the serum is derived by assuming a serum volume equal to 3% of the mass of each rabbit. Kaplan, H M and Timmons, E H. The Rabbit: a Model of the Principals of Mammalian Physiology and Surgery. Academic Press, NY (1979).

TABLE 8

Time (mins)	TTX (.mu.g/ml) Std. Error	TTX in Serum (Total .mu.g absorbed) Std. Error
0	0	0
10	0.16 +/- 0.09 (SE = 0.04)	6.39 +/- 4.61 (SE = 2.30)
20	0.33 +/- 0.16 (SE = 0.08)	14.44 +/- 6.09 (SE = 3.04)
40	0.31 +/- 0.11 (SE = 0.06)	13.92 +/- 4.06 (SE = 2.03)

Thus, after 20 minutes about 14% of the applied dose of TTX was absorbed into the serum. This persisted for at least 40 minutes.

Example 7

Topical Application of Single Dose (20 .mu.l) of 0.1 mM TTX to Abraded Rabbit Cornea

TTX (Alexis Corporation) was obtained and formulated into a 0.1 mM concentration in a pH 4.3 sodium citrate vehicle (60 mM). Dutch banded rabbits were divided into two experimental groups of five and six. After general anesthesia by intramuscular injection of a mixture of xylazine and keratin, followed by topical application of 0.5% proparacaine to each eye, a #69 Beaver blade was used to create a central epithelial defect which measured between 4 mm diameter in both eyes of each rabbit. Five rabbits received a 20 .mu.l aliquot of 0.1 mM TTX into one eye and six rabbits received 20 .mu.l of the pH 4.3 sodium citrate vehicle as a control into one eye. Experimenters were masked to the contents of each aliquot.

Corneal sensation was tested with a 4-0 silk suture mounted upon a wooden cotton tip applicator such that the suture extended 5 mm beyond the wooden end of the applicator. Because the central cornea was often rendered anesthetic following mechanical epithelial debridement, the cornea was stimulated in the mid-peripheral cornea, outside of the abraded area, with the suture to produce grossly visible

indentation of the cornea as the endpoint. Care was taken not to stimulate the eyelashes. The rabbit's response was graded in the following fashion; no blink=1, partial blink without full eyelid closure=2, full blink=3. A score of 3 indicates full responsiveness and a score of 1 indicates full local anesthesia. Corneal sensation was tested prior to administration of drugs and again at 4 and 6 hours. The rabbits were observed for changes in feeding habits, movement, respiration and alertness for 24 hours. The data are presented as the mean score of 6 rabbits/treatment group. TTX treated eyes' anesthesia scores were compared to their vehicle treated fellow eyes' anesthesia scores for statistical analysis by the Wilcoxon test. The results are shown in FIG. 8.

At 4 hours the vehicle treated eyes had an average anesthesia score of 2.67 (SD=0.52). In contrast the TTX treated eyes were significantly anesthetic with a mean score of 1.20 (SD=0.45) (p=0.0411). At 6 hours the vehicle treated eyes had a mean anesthesia score of 3.00 (SD=0.00). In contrast at 6 hours the TTX treated eyes were still significantly anesthetic with a mean anesthesia score of 2.00 (SD=1.00) (p=0.0411). Thus, one 20 .mu.l dose of 0.1 mM TTX provided at least 6 hours of local anesthesia when applied to an abraded rabbit cornea.

Example 8

Topical Application of Single Dose (20 .mu.l) of 0.2 mM TTX to Abraded Rabbit Cornea

TTX was obtained and formulated into a 0.2 mM concentration in a pH 4.3 sodium citrate vehicle (60 mM). Dutch banded rabbits were divided into two experimental groups of five and six. After general anesthesia by intramuscular injection of a mixture of xylazine and keratin, followed by topical application of 0.5% proparacaine to each eye, a #69 Beaver blade was used to create a central epithelial defect which measured between 4 mm diameter in both eyes of each rabbit. Six rabbits received a 20 .mu.l aliquot of 0.2 mM TTX into one eye and five rabbits received 20 .mu.l of the pH 4.3 sodium citrate vehicle as a control into one eye. Experimenters were masked to the contents of each aliquot.

Corneal sensation was tested with a 4-0 silk suture mounted upon a wooden cotton tip applicator such that the suture extended 5 mm beyond the wooden end of the applicator. Because the central cornea was often rendered anesthetic following mechanical epithelial debridement, the cornea was stimulated in the mid-peripheral cornea, outside of the abraded area, with the suture to produce grossly visible indentation of the cornea as the endpoint. Care was taken not to stimulate the eyelashes. The rabbit's response was graded in the following fashion; no blink=1, partial blink without full eyelid closure=2, full blink=3. A score of 3 indicates full responsiveness and a score of 1 indicates full anesthesia. Corneal sensation was tested prior to administration of drugs and again at 4, 6, and 8 hours. The rabbits were observed for changes in feeding habits, movement, respiration and alertness for 24 hours. The data are presented as the mean score of 6 rabbits/treatment group. TTX treated eyes' anesthesia scores were compared to their vehicle treated fellow eyes' anesthesia scores for statistical analysis by the Wilcoxon test. The results are shown in FIG. 9

All of the vehicle treated eyes were normal throughout the experimental period with an average anesthsia score of 3.00 (SD=0.00). In contrast the TTX treated eyes showed significant local anesthesia. At 4 hours the TTX treated eyes were significantly anesthetic with a mean score of 1.17 (SD=0.41) (p=0.0022). At 6 hours the TTX treated eyes were still significantly anesthetic with a mean anesthesia score of 1.33 (SD=0.82) (p<0.0152). At 8 hours the TTX treated eyes were still significantly anesthetic with a mean anesthesia score of 1.50 (SD=0.84) (p<0.0152). Thus, one 20 .mu.l dose of 0.2 mM TTX provided at least 8 hours of local anesthesia when applied to an abraded rabbit cornea.

Example 9

Calculation of Dosages

TTX has been determined to be an effective and non-toxic anesthetic for topical administration to partially de-epithelialized rabbit corneas for at least 6 h at a dose of 1.0 mM, or approximately 1.0-1.8% of the estimated lethal human dose. Even lower effective dosages for topical administration to partially de-epithelialized rabbit corneas were established, including 20 .mu.l aliquots of either 0.1 mM TTX or

0.2 mM TTX, corresponding to 0.6 .mu.g and 1.27 .mu.g, respectively.

While the toxic dose of TTX has been well studied in a number of animal species (Kao CY, Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. Pharm Rev 18:997-1049 (1966)), it has been only imprecisely estimated for humans. Cornish reported a case of TTX intoxication and estimated the lethal oral dose to be about 10-18 .mu.g/kg based on the amount of fish tissue consumed (Cornish J N, Susceptibility of man to pufferfish toxin. Med J Aust 2:48 (1973)). For a 70-kg person, the lethal dose would therefore be 0.7-1.26 mg. The dose lethality curve for TTX is very steep. In mice, the minimum intraperitoneal lethal dose is 8 .mu.g/kg and the LD100 is 12 .mu.g/kg (Kao CY, Fuhrman F A, Pharmacological studies on tarichatoxin, a potent neurotoxin. J Pharmacol 140:31-40 (1963)). The lethal dose for oral administration in mice is much higher, 332 .mu.g/kg (Kao CY, Pharm Rev 18:997-1049 (1966)). Because of the steep dose-lethality curve, reduction of the effective topical dose of TTX to 1% or less of the minimum lethal dose should result in a therapeutically useful dose.

More sensitive testing systems using an esthesiometer, such as the Boberg-Ans or the Cochet and Bonnet device are well-known in the field and can be used to determine safe human dosages. Such testing can help determine whether TTX can produce partial local anesthesia or analgesia at doses even lower than 1.0-1.8% of the estimated lethal human dose used in this study.

Example 10

Topical Administration of Saxitoxin (STX) to Partially De-epithelialized Rabbit Corneas

Saxitoxin (Alexis Corporation) was formulated into 1 mM concentration in balanced saline solution (BSS). Ten Dutch banded rabbits were divided into two experimental groups of five. After general anesthesia by intramuscular injection of a mixture of xylazine and keratin, followed by topical application of 0.5% proparacaine to each eye, a #69 Beaver blade was used to create a central epithelial defect which measured between 4 mm diameter in one eye of each rabbit. Five rabbits then received a 20 .mu.l aliquot of Saxitoxin into the inferior conjunctival cul-de-sac of one eye and five rabbits received a 20 .mu.l aliquot of the BSS vehicle as a control into the fellow eye. Experimenters were masked to the contents of each aliquot.

Corneal sensation was tested with a 4-0 silk suture mounted upon a wooden cotton tip applicator such that the suture extended 5 mm beyond the wooden end of the applicator. Because the central cornea was often rendered anesthetic following mechanical epithelial debridement, the cornea was stimulated in the mid-peripheral cornea, outside of the abraded area, with the suture to produce grossly visible indentation of the cornea as the endpoint. Care was taken not to stimulate the eyelashes. The rabbit's response was graded in the following fashion; no blink=1, partial blink without full eyelid closure=2, full blink=3. A score of 3 indicates full responsiveness and a score of 1 indicates full local anesthesia. Corneal sensation was tested prior to administration of drugs and again at, 2, 4, and 6 hours. The rabbits were observed for changes in feeding habits, movement, respiration and alertness for 24 hours. The data are presented as the mean score of 5 rabbits/treatment group. The results are shown in FIG. 10 (As the mean local anesthesia score +/- standard error) and again in Table 9 (as the mean anesthesia score +/- the standard deviation). Statistical analysis was by the Wilcoxon test.

TABLE 9

Time (min)	1 mM Saxitoxin	Vehicle
120	1.0 +/- 0.00	2.8 +/- 0.45
240	1.80 +/- 1.09	2.8 +/- 0.45
360	3.0 +/- 0.00	2.8 +/- 0.45

After 2 hours all of the Saxitoxin treated rabbit eyes were significantly anesthetic compared to vehicle treated eyes with a mean anesthesia score of 1.0 (SD=90)(p=0.004). In contrast the vehicle treated eyes had an mean anesthesia score of 2.8 (SD=0.45). After 4 hours the Saxitoxin treated eyes were still significantly anesthetic with a mean anesthesia score of 1.8 (SD=1.09)(p=0.0159), while the vehicle treated eyes had a mean anesthesia score of 2.8 (SD=0.45). By 6 hours the Saxitoxin treated eyes had returned to normal with a mean anesthesia score of 3.0 (SD=0), while the vehicle treated eyes had a mean anesthesia score of 2.8 (SD=0.45). None of the rabbits showed any signs of systemic toxicity. Thus, treatment of corneal abraded rabbit corneas with 20 .mu.l of 1 mM Saxitoxin provided at least 4 hours of corneal anesthesia.

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All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the invention has been described with respect to particular embodiments, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention.

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United States Patent *5,846,975*
Pan , et al. **December 8, 1998**

Use of amino hydrogenated quinazoline compounds and derivatives thereof for abstaining from drug dependence

Abstract

This invention relates to the use of amino hydrogenated quinazoline compounds and derivatives thereof, such as tetrodotoxin, for abstaining from drug dependence in human. Such compounds are administered by subcutaneous, intramuscular or intravenous injection for abstaining from drug dependence, the said drug is alkaloids and nitrogen-containing non-amino acid compound, for example opium, morphine, heroin and the like. Such compounds without drug dependence and low toxicity and side effect can abstain rapidly from drug dependence.

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Claims

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1. A method of treating drug dependence in a human which comprises administering to the human suffering from drug dependence at least one amino hydrogenated quinazoline having the general formula I in an amount effective to cause said drug dependent human to abstain from drug-dependence ##STR7## wherein R.sub.2 and R.sub.5 each is selected from the group consisting of H, OH, and OAc; R.sub.1 is selected from the group consisting of H, C.sub.1 -C.sub.4 alkyl, OH, OR, OC(O)R', NH.sub.2 NHR", and NR"R'" wherein R is C.sub.1 -C.sub.6 alkyl, R' is C.sub.1 -C.sub.3 alkyl, and R", and R'" is C.sub.1 -C.sub.4 alkyl;

R.sub.3 and R.sub.4 are .dbd.O; or

when R.sub.3 is H, R.sub.4 is selected from the group consisting of:

--ROH, wherein R is a branched or straight chain C.sub.1 -C.sub.7 alkyl,

--CH(OH)NHOMe,

--NAP--gly,

--NAP--en,

--CH.sub.2 NH.sub.2,

--CH.sub.2 NHCH.sub.3,

--AAG,

--NMAG, and

--ANT;

or when R.sub.3 is OH or OC(O)R wherein R is C.sub.1 -C.sub.3 alkyl, R.sub.4 is selected from the group consisting of

--CHO,

--CH.sub.2 --gly,

--CH.sub.2 --.beta.--Ala,

--CH.sub.2 --Lys,

--CH.sub.2 --en,

--CH.sub.2 --NAP--Lys,

--CH.sub.2 --NAP--en,

--CH(OH)CH(NH.sub.2)COOH,

--NH(CH.sub.2).sub.n COOH,

--NH(CH.sub.2).sub.n NH.sub.2 and

NH(CH.sub.2).sub.n CH(NH.sub.2)COOH

wherein:

n=1-6,

en is ethylene,

NAP is 4-triazo-2-nitrobenzoic amide,

AAG is 2-triazo-O-aminobenzoic amide,

NMAG is O-methylaminobenzoic amide,

ANT is O-aminobenzoic amide.

2. The method according to claim 1, wherein the amino hydrogenated quinazoline compounds and derivatives thereof are compounds having the following general formula II, ##STR8## wherein R.sub.1 can be selected from the group consisting of OH, H, an alkyl or a oxyalkyl with C.sub.1 -C.sub.4, NH.sub.2, NHR", NR"R'", among them R" and R'" can be an alkyl with C.sub.1 -C.sub.4.

3. The method according to claim 1, wherein the amino hydrogenated quinazoline compounds and derivatives thereof are compounds having the following general formula III, ##STR9## wherein: R.sub.3, R.sub.4 are .dbd.O, or

when R.sub.3 is H, R.sub.4 is selected from the group consisting of:

--CH.sub.2 OH,

--CH(OH)NHOMe,

--NAP--gly,

--NAP--en,

--CH.sub.2 NH.sub.2,

--CH.sub.2 NHCH.sub.3,

--AAG,

--NMAG, and

--ANT;

wherein, en, NAP, AAG, NMAG and ANT have the same definitions as stated above.

4. The method according to claim 1, wherein the amino hydrogenated quinazoline and their derivatives are compounds having the following general formula IV, ##STR10## wherein, R.sub.4 can be selected from the group consisting of: --CHO,

--CH.sub.2 --Gly,

--CH.sub.2 --.beta.--Ala,

--CH.sub.2 --Lys,

--CH.sub.2 --en,

--CH.sub.2 --NAP--Lys

--CH.sub.2 --NAP--en,

--CH(OH)CH(NH.sub.2)COOH;

--NH(CH.sub.2).sub.4 CH(NH.sub.2)COOH;

--NHCH.sub.2 COOH;

--NHCH.sub.2 CH.sub.2 COOH; and

--NHCH.sub.2 CH.sub.2 NH.sub.2,

wherein en and NAP have the same definition as stated above.

5. The method according to claim 2, wherein the group R.sub.1 of the amino hydrogenated quinazoline compounds and derivatives thereof is --OH.

6. The method according to claim 2, wherein the group R.sub.1 of the amino hydrogenated quinazoline compounds and derivatives thereof is H.

7. The method according to claim 3, wherein the group R.sub.3 of the amino hydrogenated quinazoline compounds and derivatives thereof is AAG.

8. The method according to claim 3, wherein the group R.sub.4 of the amino hydrogenated quinazoline compounds and derivatives thereof is NMAG.

9. The method according to claim 3, wherein the group R.sub.4 of the amino hydrogenated quinazoline compounds and derivatives thereof is ANT.

10. The method according to claim 3, wherein the group R.sub.3, R.sub.4 of the amino hydrogenated quinazoline compounds and derivatives thereof is .dbd.O.

11. The method according to claim 4, wherein the group R.sub.4 of the amino hydrogenated quinazoline compounds and derivatives thereof is --CHO.

12. The method according to claim 2, wherein the group R.sub.4 of the amino hydrogenated quinazoline compounds and derivatives thereof is CH(OH)CH(NH.sub.2)COOH.

13. The method according to claim 4, wherein the group R.sub.4 of the amino hydrogenated quinazoline compounds and derivatives thereof is NH(CH.sub.2).sub.4 --CH(NH.sub.2)COOH.

14. The method according to claim 4, wherein the group R.sub.4 of the amino hydrogenated quinazoline compounds and derivatives thereof is NHCH.sub.2 COOH.

15. The method according to claim 4, wherein the group R.sub.4 of the amino hydrogenated quinazoline compounds and derivatives thereof is NHCH.sub.2 CH.sub.2 COOH.

16. The method according to claim 4, wherein the group R.sub.4 of the amino hydrogenated quinazoline compounds and derivatives thereof is NHCH.sub.2 CH.sub.2 NH.sub.2.

17. A method for treating a human suffering from drug dependence, comprising administering to said human a daily dosage between 5 and 300 .mu.g of at least one compound of formula I: ##STR11## wherein R.sub.2 and R.sub.5 each is selected from the group consisting of H, OH, and OAc; R.sub.1 is selected from the group consisting of H, C.sub.1 -C.sub.4 alkyl, OH, OR, OC(O)R', NH.sub.2 NHR", and NR"R"' wherein R is C.sub.1 -C.sub.6 alkyl, R' is C.sub.1 -C.sub.3 alkyl, and R" and R"' are C.sub.1 -C.sub.4 alkyl;

R.sub.3 and R.sub.4 are .dbd.O or when R.sub.3 is H, R.sub.4 is selected from the group consisting of:

--ROH, wherein R is a branched or straight chain C.sub.1 -C.sub.7 alkyl,

--CH(OH)NHOMe,

--NAP--gly,

--NAP--en,

--CH.sub.2 NH.sub.2,

--CH.sub.2 NHCH.sub.3,

--AAG,

--NMAG, and

--ANT;

or wherein R.sub.3 is OH or OC(O)R wherein R is a C.sub.1 -C.sub.3 alkyl and R.sub.4 is selected from the group consisting of

--CHO,

--CH.sub.2 --gly,

--CH.sub.2 --.beta.--Ala,

--CH.sub.2 --Lys,

--CH.sub.2 --en,

--CH.sub.2 --NAP--Lys,

--CH.sub.2 --NAP--en,

--CH(OH)CH(NH.sub.2)COOH,

--NH(CH.sub.2).sub.n COOH,

--NH(CH.sub.2).sub.n NH.sub.2 and

--NH(CH.sub.2).sub.n CH(NH.sub.2)COOH;

wherein

n=1-6,

en is ethylene,

NAP is 4-triazo-2-nitrobenzoic amide,

AAG is 2-triazo-O-aminobenzoic amide,

NMAG is O-methylaminobenzoic amide,

ANT is O-aminobenzoic amide.

18. The method according to claim 17 wherein the method of administration is selected from the group consisting of oral, subcutaneous, intramuscular and intravenous administration.

19. The method according to claim 1, wherein said amino hydrogenated quinazoline is tetrodotoxin.

20. The method according to claim 17, wherein said amino hydrogenated quinazoline is tetrodotoxin.

21. The method according to claim 1, wherein said drug dependence is opioid drug dependence.

22. The method according to claim 17, wherein said drug dependence is opioid drug dependence.

Description

TECHNICAL FIELD

This invention is concerned with amino hydrogenated quinazoline compounds and derivatives thereof, particularly their new application for causing humans to abstain from drug dependence on alkaloids and synthetic non-amino acid nitrogen.

BACKGROUND OF THE INVENTION

The research of amino-hydro quinazoline and its derivatives originated from the knowledge for crystal tetrodotoxin(TTX).

TTX is a well-known amino-perhydro quinazoline with its molecular formula as C.sub.11 H.sub.17 N.sub.3 O.sub.8 and its molecular weight 319.27.The chemical structure for TTX is shown as following: ##STR1## TTX has been found in many puffers up to one hundred, such as tetrodontidae, diodontidae, molidae, triodontidae and other families of puffer. These puffers are classified as hard-fish sub-class, fish class. Tissues of these puffers rich in TTX include ovary, liver, skin, and gastrointestinal. Methods of TTX extracting from these tissues have been reported by T. Goto et al. (Tetrohedron, 21, 2059. 1965) and E. F. Murtha (Expte. Therap., 122, 246. 1958). For TTX synthesis method, see Y. Kishi, et al., J. Am. Chem. Soc., 94(26), 9217, 9219. 1972. Later studies revealed that TTX and its derivatives could be detected in amphibians like salamanders, in mollusks like octopus, shellfish, and snails, in arthropods like crabs, limulus, starfish and in plants like red algae. Very recently, it was found that TTX was also produced by some geneses bacteria such as vibros, pseusdomona, streptomyces josamycetinus and etc. TTX is an extreme poison whose toxicity is 1,250 times more than that of sodium cyanide. It was estimated that dosage of TTX as little as 0.5 mg could bring death of a person

with 70 kg body weight. In another report, subcutaneous injection of 300 .mu.g TTX was enough to put death of a man with 50 kg body weight.

Although TTX has been long recognized, its clinical indications limit in following aspects:

1. Analgesia

(1). TTX produces pronounced analgesic effect on various pains caused by burning, trauma, injuries from falls, fractures, contusions and strains, especially for neuragia, myalgia and arthralgia. Unless the diseases are inveterate, TTX is a powerful analgesic.

(2). Local anesthesia

TTX can be used as local anesthetic and is ten thousand-fold more powerful than those commonly used local narcotics (Kao CY and Fulman FA, J. Pharmacol., 140, 31-40, 1965). The combined preparations of TTX with widely-used local anesthetics have been published in U.S. Pat. No. 4,022,899 and U.S. Pat. No. 4,029,793.

(3). As a potent analgesic for late cancer patients. TTX exerted satisfactory effect of pain relieving on cancer pain, and no drug addiction cases were reported ›Kao C Y, Pharm. Rev. 18(2):997, 1966!

2. Sedation

(1) As antipruritic for winter skin itch, prurigo, thylacitis, itch mite and sarcoptic mite. It also facilitated recovery from these dermatosises.

(2) As asthma and pertusis abirritant.

(3) As enuresis inhabitant.

3. Antispasmodic

TTX is an effective antispasmodic for myospasm, gastrospasm and other kinds of spasms, particularly for tetanospasm.

4. Blood pressure depressor

TTX produced strong depressing effect on blood pressure, for example, administration of 2-3 .mu.g/kg TTX to the cat (i.v.) could abruptly lower its arterial pressure as much as two thirds of the normal value, and the duration of it action is fairly short, these characteristics possibly make it useful in first aid of hypertension crisis in the clinic.

5. Others

(1) TTX was reportedly effective on pain relieving for lepers (Nomiyama S: Fed. Proc.31:1 117,1972)

(2) Due to its function of congestion, TTX can elicit therapeutic effect on man's impotence and woman's asexuality.

Up to now, no applications of TTX, amino hydrogenated quinazoline compounds and derivatives thereof in abstaining from drug dependence have been reported in prior art. Here, the conception of drug dependence refers to such a physical (or physiological) state of subjects as we call it, withdrawal syndrome, which is produced by everlasting interaction between the body and drug, the symptoms include perspiration, lacrimation, yawn, rigor, goose flesh, mydriasis, vomiting, diarrhea, achycadia, hypertension, insomnia, mania, remor and etc. Subjects are obliged to use drugs continuously not for therapeutic aim but for abuse which occurs once drugtaking is stopped. Drugs here mentioned refer to alkaloids part of drugs, such as opium, morphine, cocaine, amphetamine, and other synthetic non-amino

acid nitrocompounds, such as heroin, dolantin, dihydroetorphine, methadone, and etc.

The frightening consequences of drug abuse make it desperately needed for the society to develop new de-addiction drugs which are highly effective, short of severe side reactions.

So far, the methods for addiction therapy in the world mainly rely on substitutive drugs. Briefly, they can be summarized as follows:

1. Taking small dose of opium-containing drugs. During the therapy period, opium may be gradually reduced till no opium is used in the end. This regimen lasts quite long (16 days), and its curative effect is just so-so.

2. Taking methadone orally. Methadone is used as a substitution for abused drugs like opiums. This therapy is currently applied as a standard treatment for drug abuse, most patients can free themselves from drug dependence within 10 days.

3. Using dehydroetorphine(DHE). According to clinical trials, dose-reducing therapy of DHE could alleviate abstinence symptoms within 7 to 10 days, however, during the therapy period, DHE dependence usually occurred.

4. Administration of buprenorphine.

5. Other drugs, like clinidine.

Recently, the combination of drugs above mentioned has been coming into use, for example, DHE combined with methadone was used for heroin addiction ›Sha Lijun et al. Xinyao he Linchuang (New Drugs and Clinical Remedies) 13(6), 337-339, 1994!, and also small dose of DHE combined with anisodaminum possessed the same therapeutic effect for heroin dependence ›Su Mujing et al.Chinese Drug Dependence Bulletin, 2(1): 48-51, 1992!. In addition, anisodaminum alone or plus chlorpromazine were also used for de-addiction therapy.

Among above drugs, methadone, and DHE are themselves addictive medicine if used for a long time. Anisodaminum, though lack of addictive, can produce various side effects, such as blurred vision, airway secreta drying, thirst, enuresis and etc. For these reasons, it is imperative to seek new de-addiction drugs which are highly effective, lack drug dependence and severe side reactions.

The object of the present invention is to provide a novel application of amino hydrogenated quinazoline compounds and derivatives thereof, including TTX, to cause humans to abstain from drug dependence on alkaloids.

The other object of the invention is to provide a pharmaceutical composition containing amino hydrogenated quinazoline compounds and derivatives thereof for the same therapeutic use as above.

The further object of the invention is to provide methods of causing humans to abstain from alkaloids dependence by administering amino hydrogenated quinazoline compounds and derivatives thereof to human patients.

DISCLOSURE OF INVENTION

The present invention relates to a use of amino hydrogenated quinazoline and derivatives thereof are compounds having the general formula I in the preparation of medicament for causing humans to abstain from drug-dependence, ##STR2## wherein,

R.sub.2 and R.sub.5 can be selected from the group consisting of H, OH, OAC, respectively;

R.sub.1 call be H, or an alkyl with C.sub.1 -C.sub.4, OH, OR, OC(O)R', NH.sub.2, NHR", NR"R"', among them R can be an alkyl with C.sub.1 -C.sub.6, R' can be an alkyl with C.sub.1 -C.sub.3, and R",

R'''' can be an alkyl with C.sub.1 -C.sub.4, respectively;

R.sub.3 and R.sub.4 can be .dbd.O, or

when R.sub.3 is H, R.sub.4 can be selected from the group consisting of:

--ROH, and R is a branched or straight chain alkyl with C.sub.1 -C.sub.7,

--CH(OH)NHOMe,

--NAP--gly,

--NAP--en,

--CH.sub.2 NH.sub.2,

CH.sub.2 NHCH.sub.3,

--AAG,

--NMAG, and

--ANT;

when R.sub.3 is OH or OC(O)R and R is an alkyl with C.sub.1 -C.sub.3, R.sub.4 can be selected from the group consisting of:

--CHO,

--CH.sub.2 --gly,

--CH.sub.2 --.beta.--Ala,

CH.sub.2 --Lys,

--CH.sub.2 --en,

--CH.sub.2 --NAP--Lys,

--CH.sub.2 --NAP--en,

--CH(OH)CH(NH.sub.2)COOH; and,

--NH(CH.sub.2).sub.n COOH,

--NH(CH.sub.2).sub.n NH.sub.2 ; and

--NH(CH.sub.2).sub.n CH(NH.sub.2)COOH,

wherein:

n=1-6.

en is ethylene;

NAP is 4-triazo-2-nitrobenzoic amide, indicated as formula (a);

AAG is 2-triazo-O-aminobenzoic amide, indicated as formular (b);

NMAG is O-methylaminobenzoic amide, indicated as formula (c);

ANT is O-aminobenzoic amide, indicated as formula (d); ##STR3##

Among them, three kinds of compounds with the general formula II, III, IV are preferred.

The amino hydrogenated quinazoline compounds and derivatives thereof are compounds having following general formula II, ##STR4## wherein: R.sub.1 can be selected from the group consisting of OH, an alkyl or a oxyalkyl with C.sub.1 -C.sub.4, NH.sub.2, NHR", NR"R"', among them R" and R"' can be an alkyl with C.sub.1 -C.sub.4.

Among them, the more preferred compounds are:

Tetrodotoxin R.sub.1 .dbd.OH (1);

deoxytetrodotoxin R.sub.1 .dbd.H (2);

The amino hydrogenated quiniazoline compounds and derivatives thereof are compounds having following general formula III. ##STR5## wherein:

R.sub.3, R.sub.4 are .dbd.O, or

when R.sub.3 is H, R.sub.4 is selected from the group consisting of:

CH.sub.2 OH,

CH(OH)NHOMe,

--NAP--gly,

--NAP--en,

--CH.sub.2 NH.sub.2,

--CH.sub.2 NHCH.sub.3,

--AAG,

--NMAG, and

--ANT.

Among them, the more preferred compounds are:

AAG-degradation Tetrodotoxin R.sub.4 .dbd.AAG (3);

NMAG-degradation Tetrodotoxin R.sub.4 .dbd.NMAG (4);

ANT-degradation Tetrodotoxin R.sub.4 .dbd.ANT (5); and,

degradation Tetrodotoxin R.sub.3, R.sub.4 is .dbd.O (6).

The amino hydrogenated quinazoline and their derivatives are compounds having following general formula IV, ##STR6## wherein, R.sub.4 can be selected from the group consisting of: --CHO,

--CH.sub.2 --Gly,

--CH.sub.2 --.beta.--Ala,

--CH.sub.2 --Lys,

--CH.sub.2 --en,

--CH.sub.2 --NAP--Lys

--CH.sub.2 --NAP--en,

--CH(OH)CH(NH.sub.2)COOH;

--NH(CH.sub.2).sub.4 CH(NH.sub.2)COOH;

--NHCH.sub.2 COOH;

--NHCH.sub.2 CH.sub.2 COOH; and

--NHCH.sub.2 CH.sub.2 NH.sub.2.

Among them, the more preferred compounds are:

oxytetrodotoxin R.sub.4 .dbd.CHO (7);

chiriquitoxin R.sub.4 .dbd.CH(OH)CH(NH.sub.2)COOH (8);

and the compounds with the substituted groups of R.sub.4 :

--NH(CH.sub.2).sub.4 CH(NH.sub.2)COOH (9);

--NHCH.sub.2 COOH (10);

--NHCH.sub.2 CH.sub.2 COOH (11); and,

--NHCH.sub.2 CH.sub.2 NH.sub.2 (12).

The above-mentioned amino hydrogenated quinazoline compounds and derivatives thereof, including Tetrodotoxin, could inhibit the morphine's-dependence withdrawal symptoms, and it is not addicted by the antagonists. It could effectively and promptly inhibit and allay the withdrawal symptoms and could dispel the restlessness of patients as use of these kinds of compounds with adequate dosage when the withdrawal response commences upto the most severe. The withdrawal symptoms of patients were allayed and disappeared from 5 to 30 min after accepted administration of the therapy. Patients could be quiet and almost feel numb in month, tongue and lips, however, no uneasy feeling. The numb counteracts the desire of patients for drugs, such as heroin. After several days maintenance (2-8 days, and generally 2-3 days), it reaches the clinical de-addiction which shows as the withdrawal symptoms disappeared completely and the test of morphine in urine transforms to negative. After halt in using the above-mentioned amino hydrogenated quinazoline compounds and derivatives thereof, there is no dependence to them and no adverse and side effects. As using the above-mentioned amino hydrogenated quinazoline compounds and derivatives thereof to treat patients, no case developed sicchasia, vomiting, collapse or coma as with the use of other medicines. It also has no side effects as in the treatment of methadone and DHE.

The invention further involves any composition of medicines, which contains an effective dosage of above-mentioned amino hydrogenated quinazoline compounds and their derivatives, to leave off drug dependence. The amino hydrogenated quinazoline compounds and their derivatives in this invention

could be made by now available technologies with pharmaceutically acceptable carriers, excipients and other additives to various forms, such as injections (including subcutaneous, intramuscular or intravenous) or oral form (including hypoglossal etc. However, because the orally effective dosage is much higher than (about 20 times) that of injections, injection is preferred. It is preferred to dissolve the above-mentioned amino hydrogenated quinazoline compounds and their derivatives in weak acid water solution, such as benzoic acid or acetic acid solution with pH being 4-5.

As using to clear off drug dependence, the effective dosage of above-mentioned amino hydrogenated quinazoline compounds and their derivatives is from 5 .mu.g to 300 .mu.g when injected by subcutaneous, intramuscular or intravenous.

The invention also involves any method to clear off drug dependence which uses effective dosage of above-mentioned aminoperhydroquinazoline compounds and their derivatives through oral or subcutaneous, intramuscular or intravenous injections.

Although the compounds of the invention used in clearing one off drug dependence are all severely toxic substances, the administered dosage of therapy is much lower than intravenously toxic dosage, such as the toxic dosage of TTX which is 300 .mu.g/person.

It takes 3-5 days to clear one off the drug dependence of opium, heroin, morphine, cocaine, amphetamine (ice), dolandin, dihydroetorphine and methadone and this kind of addictable alkaloid when using the aminoperhydroquinazoline compounds and their derivatives of this invention alone or co-use them with other anti-addiction medicines. They do not induce addiction themselves but could clear one off drug dependence within a short time, with little toxic and side effects, and quick recovering of health (7-10 days).

BEST MODE FOR CARRYING OUT THE INVENTION

Example 1

3 mg compound (1), TTX, was dissolved in 200 ml injection used water which containing 9.4 g benzoic acid (pH=4) and distributed it to 100 parts. Each contains compound (1) 30 .mu.g and be made as injections (1).

Example 2

15 mg compound (2), was dissolved in 200 ml pH 4 benzoic acid solution and distributed to 100 parts. Each contains compound (2) 150 .mu.g and be made as injections (2).

Example 3-5

10 mg compound (3)-(5) was dissolved in 200 ml pH 4 acetic acid individually, then divided the solution into 100 parts to prepare injections (3)-(5), so each injection contained 100 .mu.g compound (3)-(5).

Example 6

15 mg compound (6) was dissolved in 200 ml pH 4 benzoic acid, then divided the solution into 1100 parts to prepare injection (6), so each injection contained 150 .mu.g compound (6).

Example 7

3 mg compound (7) was dissolved in 200 ml pH 5 benzoic acid, then divided the solution into 100 parts to prepare injection (7), so each injection contained 30 .mu.g compound (7).

Example 8

3 mg compound (8) was dissolved in 200 ml pH 4 acetic acid, then divided the solution into 100 parts to prepare injection (8), so each injection contained 30 .mu.g compound (8).

Example 9-12

6 mg compound (9)-(12) was dissolved in 200 ml pH 5 acetic acid individually, then divided the solution into 100 parts to prepare injections (9)-(12), so each injection contained 60 .mu.g compound (9)-(12).

EXPERIMENT

Experiment 1: Comparison Experiment

Materials and Methods

1. Sample Collection

2768 cases with drug-dependence were collected in TTX de-addiction treatment, 2500 of them finished the whole process of treatment and 228 cases stopped the treatment because 206 cases could not tolerate the abstinence syndrome in the first 3 days and required to draw out this treatment, 17 cases were found in serious infectious diseases and other 5 cases were transferred to other hospitals.

These 2500 patients described above were 45-13 years old, averaged 23.5. Their drug-use period were 20-0.25 years, averaged 1.8 years. 2125 (85%) of them were male and 375 (15%) were female.

Kinds of drug-abuse: 831 cases taking Opium, 1570 cases taking Heroin, 41 cases taking Morphine, 50 cases taking DHE, 5 cases taking Dolantin, 2 cases taking Cocaine and 1 case taking Amphetamine.

Abuse routes: iv. 930, inhalation 1570.

Daily abuse amount were 5.0-0.1 g. Drug-addiction degree were preliminary divided as: Light (Group A), 1050 cases, 50 cases drawing out, 1000 cases finished the treatment of de-addiction; Medium (Group B), 1100 cases, 100 cases drawing out, 1000 cases finished the treatment of de-addiction; Serious(Group C), 568 cases,68 cases drawing out, 500 cases finished the treatment of de-addiction; Control(Group D), 60 cases randomly selected, 10 cases drawing out, 50 cases finished the treatment of de-addiction.

2. Accepted Cases Selection

2.1 Experiment Places and Case Resources

Experiment places were Health-Recovering Hospital and De-Drug-Addiction Agency recognized by China government. Accepted cases were de-addiction volunteers of drug-dependence on Opium, Morphine, Dolantin, Heroin, DHE, etc. Treatment formalities were voluntarily carried out by themselves or their families. Cases were free except drug-abuse during the treatment period.

2.2 Diagnostic Criterion

According to WHO drug-dependence definition and U.S.A DSM-III-R Criterion, the cases were diagnosed. Both their urine test and Naloxone addiction-press test were positive and their addiction syndrome were significant before TTX treatment.

3. De-Addiction Program Design

3.1 Grouping

The accepted cases were examined in detail, then according to their drug-taking period, amount, species,

physical and mental conditions, they were divided into Light, Median and Serious 3 degrees and given general, intensive and special care.

4. Methods

4.1 Observation Methods

4.1.1 According to clinical abstinence syndrome and intensity and literature criterion, the cases were divided into III grades generally.

4.1.2 Given marks by abstinence syndrome frequency before and after treatment. Grade I 2.times.5; Grade II 3.times.6; Grade III 4.times.8.

4.1.3 Given marks by adverse reactions (see the table)before and after treatment.

"1" known by requiring;

"2" could be tolerated by chief complaint;

"3" could not be tolerated, drug amount needed reducing;

"4" stopped treatment.

4.1.4 Inhibition effect was marked as the sum of the above 2 items marks. The lower the mark, the better the inhibition effect; or the worse. Accepted cases were systematically examined, included system examination. Abstinence syndrome was marked on chief complaint and physical syndrome appearance. Laboratory examinations included test of blood, urine routine, liver function, electrocardiogram, X-ray for heart and lung, and drug urine test. Physical complication, psychosis and cardiovascular diseases patients were excluded after the treatment beginning. The treatment results were observed and recorded everyday and marked by a proper person. Everyday, after drug administration, cases were measured blood pressure, heart rate and marked on the adverse reactions. Each treatment period was set as 3-8 days. Treatment efficacy was evaluated as soon as the treatment finished, and the results were statistical analysis.

4.2 Treatment Methods

Group A: TTX (from Case 1) treated;

Group B: TTX+Diazepamum treated;

Group C: TTX+Agonist treated;

Group D: Control

Among them:

Group A: Cases of drug-taking less amount, shorter period, better physical conditions and stronger will, could be only treated by TTX i.m. 1 injection per day, for 3 days.

Group B: Cases of drug-taking more amount, longer period, worse physical conditions and weaker will, could be treated by TTX with Diazepamum. In the first 3 days, i.m. or iv TTX 1-2 injections/day, bid., and iv. by drop Diazepamum 10-20 mg in 500 ml 5-10% glucose-normal saline after 4-6 hours of the first TTX administration, bid. Cases that still could not sleep were additionally administrated 50-100 mg Wintermin.

Group C: Cases of drug-taking amount more than 2 g daily, period longer than 2 years and had abuse experience of Opium, DHE, Diazepamum, Wintermin etc. Their physical and mental conditions were

worst. Their aim was generally to reduce but not to get rid of drug-abuse. For their treatment 2 methods were designed.

Method C1: On day 1 to 5, i.m. TTX bid and on day 1 to 3, add agonist such as Methadone; day 1, 30-40 mg, day 2, 20-30 mg, day 3-4, 20 mg, day 5, 10 mg p.o.. On day 6-8, symptomatic treatment.

Methods C2: On day 1-5, i.m. TTX bid, 2 hours later p.o. Diazepamum 10 mg, Clozapinum 25 mg. More serious cases were iv. by drop 50-100 mg Wintermin in 5-10% glucose-normal saline after the first TTX 2 injections. It was also suggested that p.o. estazolam 3-4 mg /day, or p.o. Clonozepam 6-10 mg/day to sustain 3-4 days then the dosage gradually reduced till day 7.

Group D: divided to 2 methods:

D1: Methadone p.o.;

D2: DHE iv. or p.o..

Daily dosage of each group see Tab. 1.

Results

1. Criterion

In this program, all observations of therapeutic efficacy were recorded in time everyday and quantified in a table to give marks, which was also referred to chief complaints to certain the criterion of treatment effect.

1.1 Significantly effective: Physical syndrome disappeared, mental syndrome alleviated obviously. Marks were lower than 25 in 72 hours and lower than 10 at the end of the treatment.

1.2 Effective: Physical syndrome and mental syndrome reduced. Marks were lower than 60 in 72 hours and lower than 25 at the end of the treatment.

1.3 Not effective: Cases had no or just slight reduction of physical syndrome. Their mental syndrome was still obvious. Marks were more than 60 in 72 hours and more than 25 at the end of the treatment, or stopping drug administration during the treatment.

2. Evaluation of Efficacy

2.1 After drug administration the treatment groups showed significant inhibition effect on the generation and reaction intensity of abstinence syndrome. The therapeutic effects were highly significantly different from control group in 72 hours and at the end of the treatment ($p<0.01$). (see Tab.2.)

2.2 Inhibition Effect on Various Intensity of Abstinence Syndrome

See Tab.3. The total syndrome marks of 6 groups were not statistically different ($p>0.05$) before the treatment, while there were significant difference in mark reduction in 72 hours treatment ($p<0.01$), but there were no difference in total mark reduction in group A,B and C1,C2 after 72 hours treatment ($p>0.05$).

2.3 Inhibition Effect on Various Abstinence Syndrome

Comparing the frequency of various abstinence syndrome before and after the treatment, treatment groups showed significant reduction in 15 min. and after 72 hours treatment, especially in Group A,B. Treatment results of various abstinence syndrome see Tab. 4.

3. Comparison of Time of Onset of Drug Effect.

All accepted cases were administrated drugs when various abstinence syndrome reached its peak (syndrome mark more than 20). Noticed the time point of abstinence syndrome significant alleviated or disappeared. The results are as fellows:

TTX i.m.	3-20 min.	Mean:	8.00 .+-. 2.00 min.
TTX iv. by drop	2-15 min.	Mean:	5.40 .+-. 1.20 min.
DHE i.m.	7-25 min.	Mean:	10.54 .+-. 4.50 min.
DHE i.v. by drop	5-20 min	Mean:	13.11 .+-. 4.12 min
Methadone p.o.	50-90 min.	Mean:	71.00 .+-. 21.86 min.

TTX showed its effect was faster than control drug whatever the administration routes.

4. Gradual Changes of Hamilton Anxiety

Results see Tab. 5.

The change tendency of Hamilton anxiety was closely related to its abstinence syndrome in each group. Mark of 6 groups was all reduced after treatment, group A,B,C 1 ,C2 was plateaued; on day 4,5, Group D2 elevated then reduced gradually; while Group D1 was elevated on day 8-10 after administration stopping. All of Group A,B and C showed significantly different from Group D ($p<0.05$ or $p<0.01$).

5. Drug Adverse Reactions

No adverse reactions were found in Group A,B,C 1,C2,D1 and D2. No abnormality was found in blood, liver, kidney function and electrocardiogram before and after treatment. Urine drug amount and Naloxone addiction-press test were all negative after the treatment.

Most cases in Group A treated with TTX had the feeling of numbness in tong tip and lips. So did small number of group B and only a few of group C.

Some cases in Group D2, D1 treated with DHE and Methadone had the syndrome of dizzle, cardiopalmus, nausea and hidrosis etc.

Almost every serious cases felt systematic pain, tiredness at the later phase of the de-abstinence treatment.

6. De-experiment Rate

Some cases drew out the experiment during the treatment process. The rate in Group A was about 5%, Group B 10%, Group C1 12%, Group C2 15%, Group D1 15%, Group D2 35%.

Experiment 2: Typical Cases

Cases 1-20

Tab. 6 showed of the sex, drug-dependent species, drug-taking period and routes of typical case 1-20, and the treatment dosage and days of administration of the injections (Example 1-8) according to the present invention. Tab. 7 showed cases abstinence syndrome before treatment, including subjective and objective syndrome. Tab. 8 showed de-addiction situations after the compounds administration 5-15 min. and 4 days. It suggested that the compounds according to the present invention could get rid of addiction rapidly with less side effects.

Industrial Applicability

In this invention the discovery of new uses for the titled compounds suggests that this type of compounds can be used to prepare drugs for treatment of human drug-dependence.

TABLE 1

Daily Dosage of TTX and Control Group

Administration / Days	Group A TTX, im, .mu.g n = 1000	Group B TTX, im, .mu.g n = 1000	Group C C1 TTX, im/iv, .mu.g	Group C C2 TTX, im/iv, .mu.g	Group D D1 Methadong, po, mg	Group D D2 DHE, .mu.g
d1	30	60	60 + Methadone 30 mg po	60 + Diazepamum 10 mg + Clorozapimum 25 mg	30	400 iv
d2	30	60 + Diazepamum 10-20 mg in 500 ml Glucose + N.S	60 + Methadone 30 mg po	60 + Diazepamum 10 mg + Clorozapimum 25 mg	30	400 iv
d3	30	60 + Diazepamum 10-20 mg in 500 ml Glucose + N.S	60 + Methadone 30 mg po	60 + Diazepamum 10 20 mg po Clorozapimum 25 mg	30	400 iv
d4		60	60 + Methadone 30 mg po	60 + Diazepamum 10 mg + Clorozapimum 25 mg	20	350 po
d5		30	30 + Methadone 10 mg po	60 + Diazepamum 10 mg + Clorozapimum 25 mg	15	300 po
d6		30	30 +	30 +	15	200 po

	Methadone 10 mg po	Diazepamum 10 mg		
d7	30	30 + Diazepamum 10 mg	10	150 po
d8			5	100 po

TABLE 2

Comparison of Curative Effect between Treatment Groups and Control Group

Group	Case Number	Efficacy in 72 h: Significant effective	Effective	Not effective	Final Efficacy: Significant effective	Effective	Not effective	Effective Rate (%)
A	1000	970	30	0	950	50	0	100
B	1000	750	240	10	510	488	2	98.8
C1	250	100	125	25	75	170	5	98
C2	250	80	120	50	60	183	7	97.2
D1	20	10	8	2	5	14	1	95
D2	20	12	6	2	6	13	1	95

TABLE 3

Changes of Evaluating Maks on Withdrawal Syndrome

Date	Group A	Group B	Group C C1	C2	Group D D1	D2
before	10.0 .+-. 0	16.5 .+-. 2.3	25.4 .+-. 3.2	25.5 .+-. 3.3	17.4 .+-. 7.9	17.4 .+-. 2.0
treatment						
d1	5.0 .+-. 1.5	11.6 .+-. 1.4	16.8 .+-. 4.2	12.7 .+-. 2.9	11.0 .+-. 3.0	5.1 .+-. 3.2
d2	3.5 .+-. 1.5	8.3 .+-. 1.9	9.6 .+-. 2.5	9.9 .+-. 2.5		

					10.0 .+-. 2.9	4.5 .+-. 3.3
d3	1.9 .+-. 1.3	6.0 .+-. 2.0	9.5 .+-. 2.3	8.0 .+-. 2.7	8.1 .+-. 2.6	3.3 .+-. 2.5
d4		4.4 .+-. 1.8	7.1 .+-. 1.3	6.2 .+-. 2.1	7.3 .+-. 2.3	6.5 .+-. 3.7
d5		1.6 .+-. 1.5	5.1 .+-. 1.7	5.2 .+-. 1.8	5.5 .+-. 2.1	5.5 .+-. 2.9
d6			4.3 .+-. 1.4	4.3 .+-. 1.7	4.4 .+-. 1.5	4.3 .+-. 1.2
d7			1.6 .+-. 1.4	1.6 .+-. 1.5	2.1 .+-. 1.6	3.1 .+-. 1.5
d8			1.3 .+-. 1.5	1.3 .+-. 1.4	4.5 .+-. 1.0	2.8 .+-. 1.7

TABLE 4

Evaluating Marks on Withdrawal Syndrom before and after Treatment

Withdrawal syndrome	Group A before (15 min) after 72 hrs	Group B before (15 min) after 72 hrs	Group C1 before (15 min) after 72 hrs	Group C2 before (15 min) after 72 hrs	Group D1 before (15 min) after 72 hrs	Group D2 before (15 min) after 72 hrs
Lacrimation	983(81)1	985(180)120	246(210)171	245(208)71	19(20)17	18(3)19

Salivation
980(40)31
1000(112)101
250(167)198
250(200)77
20(20)18
19(3)20
Yawns 921(450)21
999(720)57
250(172)98
250(198)42
20(20)16
20(10)20
Insomnia
998(350)98
998(620)512
250(160)198
25(220)72
19(19)17
19(18)18
Gooseflesh
921(80)2
958(120)81
245(42)25
246(41)41
17(19)18
18(18)18
Vomiting
142(0)0
167(34)11
47(40)3
50(41)0
7(8)8
4(1)8
Nausea
640(0)0
941(100)20
236(41)7
225(40)3
I5(17)15
14(7)11
Anorexia
945(400)41
958(801)122
241(202)51
240(240)230
18(19)20
19(17)19
Anxiet and
998(151)123
998(670)210
250(240)50
250(210)17
19(20)19
18(17)20
restlessness
Systemic
888(101)140
966(121)720
248(240)247
241(240)247
17(19)18
17(13)18
Pain
Abdominal-
850(32)21
900(401)126
247(128)33
235(131)17
I5(16)14

16(16)17
gia &
diarrhea
Muscular
683(121)90
590(252)257
130(60)57
150(48)12
11(11)13
10(13)17
tremors
Rapid pulse
830(71)3
835(307)123
213(200)35
215(147)35
10(14)18
10(14)19
Cold & hot
958(21)0
960(25)3
249(120)7
245(98)8
18(20)19
17(14)18
Pupil (mm)
2.8 .+-. 0.3
2.9 .+-. 0.8
2.3 .+-. 0.5
2.8 .+-. 0.5
2.5 .+-. 0.4
2.4 .+-. 0.7
1.4 .+-. 0.8
1.8 .+-. 0.5
1.3 .+-. 0.7
1.9 .+-. 1.0
1.9 .+-. 1.1
1.7 .+-. 0.7
Weakness
767(667)672
880(701)8.08
245(245)246
225(224)248
15(17)19
18(7)19
Thirsting for
999(450)720
1000(661)881
250(249)125
250(230)247
20(20)20
20(19)20

DSM-III-R Diagnostic Criterion

TABLE 5

Changes of the Evaluating Marks (HAMA) on Anxiety Symptom
Group A
Group B
Group C1
Group C2
Group D1
Group D2
n = 1000
n = 1000
n = 250
n = 250
n = 20

n = 20

Date	X .+-. S	X .+-. S	X .+-. S	X .+-. S	X .+-. S	X .+-. S
before treatment	22 .+-. 4	24 .+-. 5	25 .+-. 5	22 .+-. 4	22 .+-. 4	22 .+-. 4
after treatment	11 .+-. 5	13 .+-. 6	11 .+-. 5	18 .+-. 5	11 .+-. 5	3.3 .+-. 2.5
d1						
d2	8 .+-. 4	11 .+-. 5	8 .+-. 5	11 .+-. 5	8 .+-. 5	3.2 .+-. 2.5
d3	7 .+-. 3	10 .+-. 4	5.1 .+-. 2.8	10 .+-. 4	5 .+-. 3	2.3 .+-. 1.5
d4	4 .+-. 3	8 .+-. 4	4.8 .+-. 2.8	7 .+-. 3	4.8 .+-. 3	8 .+-. 5
d5	3 .+-. 2	4 .+-. 2	4 .+-. 3	5 .+-. 7	4 .+-. 3	6 .+-. 3
d6		3 .+-. 2	4 .+-. 3	4 .+-. 3	0 .+-. 3	0.7 .+-. 2.7
d7			6.4 .+-. 2.2	3.5 .+-. 2.5	5.3 .+-. 2	4.9 .+-. 2.6
d8			7.0 .+-. 2.7	3 .+-. 2.5	4 .+-. 2.5	4.5 .+-. 3.0

TABLE 6

Dosage of the Compound According to the Present Invention for De-addiction
(Treatment Results of 20 Typical Cases)

Case No.	Sex	Drug type	Using Drug Period (year)	Drug Dosage (g/day)	Drug route of use	De-addiction Comp.	Dosage	Administration Route	Days of use
1	male	Herion	4	0.2	iv	(1)	30 .mu.g	im	5 .times. 10
2	male	Opium	4	0.4	inhalate	(2)	150 .mu.g	im	3 .times. 8
3	male	Opium	1	0.4	inhalate	(3)	100 .mu.g	im	2 .times. 5
4	male	Herion	7 months	1.0	inhalate	(4)	100 .mu.g	im	3 .times. 4
5	male	Herion	3	1.0	inhalate	(5)	100 .mu.g	im	4 .times. 8
6	male	Herion	1	1.0	inhalate	(6)	150 .mu.g	im	3 .times. 5
7	female	Herion	1.6	0.8	iv	(7)	30 .mu.g	iv	6 .times. 8
8	male	Opium	1	0.2	inhalate	(8)	30 .mu.g	im	3 .times. 7
9	male	Opium	4	0.8	inhalate	(9)	60 .mu.g	im	4

									.times. 6
10	male	Herion	4	1.0	inhalate	(10)	60 .mu.g	im 3	.times. 6
11	male	Herion	5	0.4	inhalate	(11)	60 .mu.g	im 3	.times. 10
12	male	Herion	4	1.0	inhalate	(12)	60 .mu.g	im 6	.times. 12
13	male	Herion	5	1.2	iv	(8)	30 .mu.g	iv 4	.times. 11
14	male	Herion	9	0.5	inhalate	(11)	60 .mu.g	im 4	.times. 4
15	male	Herion	5	1.5	iv	(12)	60 .mu.g	iv 4	.times. 13
16	male	Herion	1	0.5	iv	(10)	60 .mu.g	im 3	.times. 5
17	male	Opium	5	1.5	inhalate	(2)	150 .mu.g	im 5	.times. 5
18	male	Opium	20	1.2	inhalate	(3)	100 .mu.g	im 5	.times. 3
19	male	Herion	2	2.5	inhalate	(1)	30 .mu.g	im 7	.times. 7
20	male	Herion	5	3.5	iv	(1)	30 .mu.g	iv 6	.times. 8

TABLE 7

Withdrawal Syndrome of the Patients before Using the Compound of the Present Invention

Case No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Subjective symptom																				
Agitating	+	+	+	+	+	+	+		+	+	+	+	+	+		+	+	+	+	+
Joint pain		+	+	+		+		+	+	+	+	+		+	+					
Cold	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+
Insomnia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Exciting	+		+					+					+							
Thirsty	+	+	+		+					+		+		+		+		+		
Dyspnea																				
Headache		+			+				+	+				+				+		+
Abdominal pain	+		+		+	+	+	+			+	+		+		+	+		+	+
Nausea		+	+	+		+	+	+			+	+		+		+	+		+	+
Lower extremity sore	+			+	+		+	+		+		+	+			+	+	+		
Thoracic depress		+			+			+		+			+	+						+
Stomachache	+		+		+		+													+
Objective symptom																				
Yamns	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin itch	+	+		+				+				+	+			+	+		+	+
Lacrimation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Finger-tremors		+			+	+		+	+	+				+			+			
Rhinorrhea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mydriasis	+		+							+						+				+
Miosis		+																		
Vomiting		+	+	+		+		+							+	+	+		+	+
Dysentery	+				+		+				+									
Nausea		+		+		+	+			+	+			+				+		+
Salivation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Toss about	+	+					+							+		+				+
Feet agitating				+	+															
Borborygmi	+	+							+					+		+		+		
Cough		+	+	+	+	+										+				
Disturbance			+				+		+					+						
Groan	+	+					+				+			+						
Diaphoresis	+																			
Proteinuria																				

```
         + +   + + +       + +        +     +    +
                                            +
Respirating
         2 2 1 2 2 2 2 2 2 2 1 2 2 2 2 2 2 2    2
                                            2
number/min
         0 0 8 0 0 0 0 0 0 0 8 0 0 0 0 0 0 0    0
                                            0
Pulse    8 8 8 8 8 9 9 8 8 8 8 7 8 8 7 9 9 8    8
                                            8
         8 4 0 8 2 0 0 4 8 4 0 2 4 2 6 0 0 4    4
                                            8
Blood pressure
         9 1 1 9 9 9 9 1 9 9 1 1 1 9 9 1 9 9    9
                                            9
         0/
           0 0 0/
                 0/
                   0/
                     0/
                       0 6/
                           0/
                             0 0 0 0/
                                     6/
                                       0 0/
                                          0/    0/
                                           0
         6 0/
             0/
               8 6 6 6 0/
                         7 6 0/
                               0/
                                 0/
                                   6 6 0/
                                          6 6    8
                                            /
         0 7 7 0 0 0 0 7 0 0 7 8 7 0 8 7 0 0    0
                                            7
           0 0         0     0 8 0     0        0
```

TABLE 8

```
Efficacy of De-addiction of the Patients after Using the Compounds of
the
Preseng Invention
After injecting the Compound (5-15 min)
   Pure numbness
          Pure numbness
                        Pulse rate (no significant
                                    d4
   in tongue,
          in arms and
                 Subjective
                      changes in blood pressure
                                    Morphine
Case
   month and lips
          legs   sense
                      after injection)
                                    in urine

 1 (+)           fine 74          (-)
 2 (+)    (+)    fine 78          (-)
 3 (+)    (+)    fine 78          (-)
 4 (+)           not fine
                      86          (-)
 5 (+)    (+)    fine 80          (-)
 6 (+)           fine 86          (-)
```

7	(+)		fine 86	(-)
8	(+)		fine 80	(-)
9	(+)		fine 82	(-)
10	(+)	(+)	fine 80	(-)
11	(+)		fine 76	(-)
12	(+)		fine 70	(+)
13	(+)	(+)	fine 80	(-)
14	(+)		fine 78	(-)
15	(+)		fine 74	(+)
16	(+)	(+)	fine 86	(+)
17	(+)		fine 84	(-)
18	(+)		fine 80	(-)
19	(.+-.)	(.+-.)	fine 84	(-)
20	(+)		fine 84	(+)

* * * * *

(4)

United States Patent *4,029,793*
Adams , et al. **June 14, 1977**

Synergistic local anesthetic compositions

Abstract

A local anesthetic composition comprising a mixture in a pharmaceutically acceptable carrier of a particular toxin, namely, tetrodotoxin or desoxytetrodotoxin, and another compound, generally a conventional local anesthetic compound or a similar compound having nerve-blocking properties.

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U.S. Class:	**424/251**; 424/267
Intern'l Class:	A61K 031/505
Field of Search:	424/251,267

References Cited [Referenced By]

U.S. Patent Documents

Other References

chemical Abstracts, vol. 69 (1968) p. 9494w.
Merck Index, 8th Ed., 1968, pp. 644 and 657.

Primary Examiner: Turner; V. D.
Attorney, Agent or Firm: Brumbaugh, Graves, Donohue & Raymond

Parent Case Text

This application is a division of application Ser. No. 369,302, filed June 12, 1973 (now U.S. Pat. No. 3,966,934), which application Ser. No. 369,302 is a continuation-in-part application of application Ser. No. 206,181, filed Dec. 8, 1971 (now abandoned), which application Ser. No. 206,181 is a continuation-in-part application of application Ser. No. 109,942 filed Jan. 26, 1971 (now abandoned).

Claims

1. An injectable local anesthetic composition having long-lasting local anesthetic effect which is a solution consisting essentially of a pharmaceutically acceptable carrier having dissolved therein

a. a heterocyclic aminoacyl anilide local anesthetic compound in a concentration of from 0.05 to 5% by weight of the carrier and

b. a toxin selected from the group consisting of from 0.5 to 10 micrograms of tetrodotoxin per milliliter of the carrier and from 10 to 20 micrograms of desoxytetrodotoxin per milliliter of the carrier.

2. The composition as defined by claim 1 wherein said component (b) is tetrodotoxin.

3. The composition as defined by claim 1 wherein said component (b) is desoxytetrodotoxin.

4. The composition as defined by claim 2 wherein the heterocyclic aminoacyl anilide is bupivacaine.

5. The composition as defined by claim 2 wherein the heterocyclic aminoacyl anilide is mepivacaine.

6. The composition as defined by claim 1 which further contains an effective amount of a vasoconstrictor.

7. A method of inducing anesthesia in mammals comprising administering to the mammal to be anesthetized an effective amount of an injectable local anesthetic composition having long-lasting local anesthetic effect which is solution consisting essentially of a pharmaceutically acceptable carrier having dissolved therein

a. a heterocyclic aminoacyl anilide local anesthetic compound in a concentration of from 0.05 to 5% by weight of the carrier and

b. a toxin selected from the group consisting of from 0.5 to 10 micrograms of tetrodotoxin per milliliter of the carrier and from 10 to 20 micrograms of desoxytetrodotoxin per milliliter of the carrier.

8. The method as defined by claim 7 wherein said component (b) is tetrodotoxin.

9. The method as defined by claim 7 wherein said component (b) is desoxytetrodotoxin.

10. The method as defined by claim 8 wherein the heterocyclic aminoacyl anilide is bupivacaine.

11. The method as defined by claim 8 wherein the heterocyclic aminoacyl anilide is mepivacaine.

12. The method as defined by claim 7 wherein said composition further contains an effective amount of a vasoconstrictor.

Description

The present invention relates to a novel anesthetic composition comprising a mixture of (1) tetrodotoxin or certain derivatives thereof and (2) another compound, generally a conventional local anesthetic compound, or a similar compound having nerve-blocking properties. The invention also relates to a process for preparing the novel anesthetic compositions and to their use for inducing anesthesia.

Toxins from marine sources of extraordinary potency have been known for many years. This application particularly concerns novel uses for tetrodotoxin.

Tetrodotoxin is obtained from the ovaries and eggs of several species of puffer fish of the suborder Gymnodontes. It is also found in certain species of California newts of the genus Taricha; and the toxin obtained from these species, known as tarichatoxin, is identical with tetrodotoxin. Tetrodotoxin has been purified, and its molecular structure is determined to be an amino perhydroquinazoline of the formula: ##STR1##

Tetrodotoxin and species in which it occurs are more fully described in Pharmacological Reviews, Vol. 18, No. 2, at pages 997-1049.

Experiments with isolated nerves have shown that tetrodotoxin behaves in a fundamentally different manner from local anesthetics such as procaine and cocaine. In a voltage-clamped giant axon from the squid or lobster, the latter agents reduce both inward initial sodium current and outward potassium

current. With tetrodotoxin, however, inward sodium current can be reduced or even obliterated, while the outward potassium current is totally unaffected. There are few, if any, other substances in which this unique action has been established.

Tetrodotoxin has not found any practical use as an anesthetic. While the compound can be used to induce nerve blocks in laboratory animals, the anesthetic dose is slightly below the lethal dose, which precludes, as a practical matter the use of the compound as an anesthetic in its own right.

Quite surprisingly, combinations of tetrodotoxin with a local anesthetic compound have been found to possess unusual anesthetic properties. This is manifested most significantly in improved longevity of action of combinations of the toxin with local anesthetics. In these combinations, tetrodotoxin is used in concentrations below that which produces reliable nerve blocks, and well below the toxic level.

Investigation of a wide variety of local anesthetics has shown that the action of the foregoing toxin in increasing longevity of action is general. Local anesthetics may be classified by characteristic chemical type. Within each chemical type there may be unexplained variations of activity. However, in all cases investigated, each member of the groups investigated has behaved similarly when combined with the foregoing toxin. Specific classes of local anesthetics investigated include anesthetic compounds characterized by

(i) the aminoacylanilide group, such as lidocaine, prilocaine, bupivacaine, mepivacaine and related local anesthetic compounds having various substituents on the ring system or amine nitrogen;

The following three ester types (ii), (iii) and (iv):

(ii). the aminoalkyl benzoate group, such as procaine, chloroprocaine, propoxycaine, hexylcaine, tetracaine, cyclomethycaine, benoxinate, butacaine, proparacaine, and related local anesthetic compounds;

(iii). cocaine and related local anesthetic compounds;

(iv). the amino carbamate group such as diperodon and related local anesthetic compounds;

(v). the N-phenylamidine group, such as phenacaine and related local anesthetic compounds;

(vi). the N-aminoalkyl amide group, such as dibucaine and related local anesthetic compounds;

(vii). the aminoketone group, such as falicain, dyclonine and related local anesthetic compounds; and

(viii). the aminoether group, such as pramoxine, dimethisoquine, and related local anesthetic compounds.

In each of the foregoing classes of local anesthetic compounds representative members have been enumerated. The experimental data support the conclusion that the observed effect of the toxin tested of unexpectedly extending the duration of action extend to the other known local anesthetic compounds of these groups and to the obvious modifications of the local anesthetic compounds tested. It may also be anticipated in the light of these discoveries that the novel combinations of the present invention will permit the use of concentrations of conventional local anesthetics in concentrations below the concentrations normally employed clinically. Thereby toxic manifestations sometimes observed as side effects can be minimized.

The chemical structures of some of the foregoing compounds are:

```
lidocaine
                ##STR2##
procaine
```

##STR3##
chloroprocaine
##STR4##
propoxycaine
##STR5##
hexylcaine
##STR6##
cocaine
##STR7##
tetracaine
##STR8##
##STR9##
cyclomethycaine
##STR10##
##STR11##
benoxinate
##STR12##
##STR13##
butacaine
##STR14##
proparacaine
##STR15##
##STR16##
diperodon
##STR17##
phenacaine
##STR18##
dibucaine
##STR19##
bupivacaine
##STR20##
mepivacaine
##STR21##
prilocaine
##STR22##
falicain
##STR23##
##STR24##
pramoxine
##STR25##

Other local anesthetic compounds which may be used in combination with tetrodotoxin (TTX) are the aminoacyl anilides described in the following table.

Table A

##STR26##

Compound	R	R.sup.1	R.sup.2	R.sup.3
A 2-tert. Butylamino-2',6'-acetoxylidide	H	H	H	C(CH.sub.3).sub.3
B 2-(N-n-Butyl-tert. butylamino)-2',6'-acetoxylidide	H	H	n-C.sub.4 H.sub.9	C(CH.sub.3).sub.3
C 2-(N-n-Propyl-tert. amylamino)-2',6'-acetoxylidide	H	H	n-C.sub.3 H.sub.7	C(CH.sub.3).sub.2 C.sub.2 H.sub.5
D 2-tert. Butylamino-2',6'-propionoxylidide	H	CH.sub.3	H	C(CH.sub.3).sub.3

E	2-(N-Ethyl-iso-propylamino)-2',6'-propionoxylidide	H	CH.sub.3	C.sub.2 H.sub.5	CH(CH.sub.3).sub.2
F	2-Methylamino-4'-(n-butoxy)-2',6'-dimethylpropion-anilide	n-C.sub.4 H.sub.9 O	CH.sub.3	H	CH.sub.3
G	2-(N-Methyl-n-propylamino)-2',6'-butyroxylidide	H	C.sub.2 H.sub.5	CH.sub.3	n-C.sub.3 H.sub.7
H	2-Dimethylamino-2',6'-acetoxylidide	H	H	CH.sub.3	CH.sub.3
J	2-Ethylamino-2',6'-acetoxylidide	H	H	H	C.sub.2 H.sub.5
K	2-Cyclobutylamino-2',6'- acetoxylidide	H	H	H	##STR27##
L	2-tert. Amylamino-2',6'-acetoxylidide	H	H	H	C(CH.sub.3).sub.2 C.sub.2 H.sub.5
M	2-(N-Methyl-n-butylamino)-2',6'-acetoxylidide	H	H	CH.sub.3	n-C.sub.4 H.sub.9
P	2-(N-Ethyl-sec. butylamino)-2',6'-acetoxylidide	H	H	C.sub.2 H.sub.5	CH(CH.sub.3)C.sub.2 H.sub.5
Q	2-Amino-2',6'-propionoxylidide	H	CH.sub.3	H	H
S	2-(N-Ethyl-n-propylamino)-2',6'-butyroxylidide	H	C.sub.2 H.sub.5	C.sub.2 H.sub.5	n-C.sub.3 H.sub.7
T	2-Diethylamino-2',6'-valeroxylidide	H	n-C.sub.3 H.sub.7	C.sub.2 H.sub.5	C.sub.2 H.sub.5

In the present invention the foregoing local anesthetics are used in a pharmaceutically acceptable carrier, such as water, water-ethanol, dextrose solutions, saline solution and blends thereof, in concentrations which are customarily used by physicians. Exemplary concentrations of local anesthetics having clinical application are:

	% by weight		
lidocaine	0.5	-	5
prilocaine	0.5	-	5
procaine	0.5	-	5
tetracaine	0.1	-	1
bupivacaine	0.25	-	1
hexylcaine	0.5	-	2.5
2-[N-n-propyl-tert. amylamino]-2',6'-acetoxylidide	0.1	-	2.0
2-[N-n-butyl-tert. butylamino]-			

2',6'-acetoxylidide	0.1	-	2.0

As mentioned above, the present invention also may permit the use of the usual local anesthetics in a lower than normal concentration. For example, the combination of tetrodotoxin with lidocaine permits the latter to be used in a concentration of as little as 0.05 percent by weight.

The carrier additionally contains from 0.5 to 10, usually from 0.5 to 5, micrograms per milliliter of tetrodotoxin or from 10 to 20 micrograms per milliliter of desoxytetrodotoxin. In addition, the local anesthetic preparation may contain a vascoconstrictor, as is well known in the art, such as epinephrine, norepinephrine, phenylephrine and levonordephrine.

The local anesthetic compositions may be prepared by dissolving the local anesthetic compound, tetrodotoxin or derivative thereof and a vascoconstrictor, when present, in the carrier or in separate portions of the carrier which are thereafter blended together.

Application of the local anesthetic compositions is accomplished in the usual manner, i.e., by infiltration or injection.

EXAMPLE 1

Female Charles River rats, weighing between 100 and 200 grams, were used. These were 5 rats per group and each animal received 0.2 milliliters of drug solution in the right thigh. The injections were made in such a way as to deposit the solution around the sciatic nerve trunk close to the popliteal space. After being injected, each animal was examined at intervals to determine onset, depth, and duration of nerve block as manifested by impairment of motor function in the injected leg. Frequencies of (a) complete block, (b) partial block, and (c) slight effect on motor function were noted for each group of animals. Two end points for duration of block were used: recovery of the ability to grasp when placed on an inclined screen and complete recovery of motor function.

All solutions contained 1 to 100,000 parts epinephrine which was added immediately prior to use. All solutions were freshly prepared on the day of use.

The results are summarized in Tables I-III. Depression was occasionally noted, but there were no fatalities with these doses of tetrodotoxin.

Table I: At 1 .mu.g/ml and 2 .mu.g/ml tetrodotoxin produced no complete blocks. At 5 .mu.g/ml, it produced complete blocks in all five legs injected. Mean onset time was about 20 minutes, and the blocks persisted for somewhere between 51/2 hours and 24 hours. All animals were completely recovered when examined 22 to 24 hours post injection. Because this concentration of tetrodotoxin by itself produced 100 percent frequency and blocks of such long duration, there are no differences, except in onset times, between the results obtained with it alone and those obtained with the tetrodotoxin-lidocaine combination. However, the combinations of 1 .mu.g/ml and 2 .mu.g/ml of tetrodotoxin with lidocaine clearly show durations of block that are markedly greater than those obtained with lidocaine alone.

TABLE I

RAT SCIATIC NERVE BLOCKS

Compound	Concentration as Base	pH	Onset (min.)	Frequency	Duration (min.) Mean .+-. S.D.

Compound	Concentration	pH	Onset	C	P	S	C.R.	R.G.
Tetrodotoxin								
	1 .mu.g/ml	4.4	--	0/5	1/5	4/5	--	--
	2 .mu.g/ml	5.4	--	0/5	2/5	3/5	--	--
	5 .mu.g/ml	4.3	22	5/5	--	--	--	5.5<24 hrs
Lidocaine								
	0.125%	5.1	8	5/5	--	--	85 .+-. 2	84 .+-. 1.5
	0.25%	5.0	5	5/5	--	--	108 .+-. 22	99 .+-. 24
Combinations								
T/L	1/0.125	4.9	5.5	5/5	--	--	309 .+-. 17	251 .+-. 51
T/L	2/0.125	4.8	5.0	4/5	1/5	--	316 .+-. 33	290 .+-. 46
T/L	5/0.125	4.6	3.5	5/5	--	--	--	6<24 hrs.
T/L	1/0.25	4.7	4.5	5/5	--	--	5.5<24 hrs.	299 (2)
T/L	2/0.25	4.8	3.0	5/5	--	--	--	5.5<24 hrs.
T/L	5/0.25	4.6	1.5	5/5	--	--	--	6< 24 hrs.

NOTES: C = Complete block; P = Partial block; S = Slight effect; R.G. = Recovery of grasping; C.R. = Complete Recovery; T = Tetrodotoxin; L = Lidocaine. Durations are for complete blocks only. Onset times are approximate. The pH's are after addition of epinephrine; all solutions contained 1:100,000 epinephrine. Numbers of blocks are in specific instances shown in parentheses.

Table II: As in the first study, 1 .mu.g/ml of tetrodotoxin produced no complete blocks; however, 2 .mu.g/ml produced a complete block, with a duration of about 2 hours, in one out of five injections. The frequency of block with 3 .mu.g/ml was only two out of five, but the block persisted for between 5 and 24 hours. In this study lower concentrations of lidocaine were used in order to ascertain whether or not the combinations show better frequencies than either tetrodotoxin or lidocaine alone.

TABLE II

RAT SCIATIC NERVE BLOCKS

Compound	Concentration as Base	pH	Onset (min.)	Frequency C	P	S	Duration (min.) Mean .+-. S.D. C.R.	R.G.
Tetrodotoxin								
	1 .mu.g/ml	4.6	--	0/5	0/5	5/5	--	--
	2 .mu.g/ml	4.7	31	1/5	0/5	4/5	120	102
	3 .mu.g/ml	4.5	56	2/5	0/5	3/5	--	5<24 hrs.
Lidocaine								
	0.05%	4.6	--	0/5	2/5	3/5	--	--
	0.1%	4.6	43	2/5	2/5	1/5	58	44
Combinations								
T/L	1/0.05	4.6	31	1/5	2/5	2/5	68	48
T/L	2/0.05	4.4	10	2/5	2/5	1/5	255	176
T/L	3/0.05	4.5	16	3/5	2/5	--	61/2<24 hrs.	

								359 .+-. 42
T/L	1/0.1	4.5	11	2/5	3/5	--	144	93
T/L	2/0.1	4.6	6	4/5	0/5	1/5	242 .+-. 68	188 .+-. 83
T/L	3/0.1	5.2	14	4/5	1/5	--	304 (1) 61/2<24 hrs. (3)	317 .+-. 50

See Notes under Table I.

Table III: Tetrodotoxin at 3 .mu.g/ml produced in three out of five animal blocks that lasted between 4 and 24 hours. In combinations with several local anesthetic agents, frequency was improved and onset times were shorter than with tetrodotoxin alone. All the combinations containing 1 .mu.g/ml of tetrodotoxin exhibited durations of block much greater than obtained with the local anesthetic agents alone. The study clearly demonstrates that, in rat sciatic nerve blocks, the presence of concentrations of tetrodotoxin that by themselves are subthreshhold can cause marked increases in the durations of block of several local anesthetic agents.

TABLE III

RAT SCIATIC NERVE BLOCKS

Compound	Concentration as Base	pH	Onset (min.)	Frequency C	P	S	Duration (min.) Mean .+-. S.D. C.R.	R.G.
Tetrodotoxin								
	1 .mu.g/ml	4.6	--	0/5	1/5	4/5	--	--
	3 .mu.g/m.	4.3	48	3/5	2/5	--	4<24 hrs.	
Lidocaine								
	2.0%	4.4	2.0	5/5	--	--	172 .+-. 17	160 .+-. 12
T/L	1/2.0	4.5	1.5	5/5	--	--	223(2) 41/2<24 hrs. (3)	188(2)
T/L	3/2.0	4.3	1.5	5/5	--	--	41/2<24 hrs.	
Bupivacaine								
	0.5%	5.0	2.5	5/5	--	--	232 .+-. 39	183 .+-. 18
T/B	1/0.5	5.2	6.0	5/5	--	--	282 (2) 5<24 hrs. (3)	265 .+-. 45
T/B	3/0.5	5.4	2.5	5/5	--	--	5<24 hrs.	
Prilocaine								
	2.0%	5.0	2.5	5/5	--	--	153 .+-. 16	123 .+-. 6
T/Pr	1/2.0	4.8	<1.0	5/5	--	--	5<24 hrs.	251 .+-. 26
T/Pr	3/2.0	4.8	2.0	5/5	--	--	5<24 hrs.	
Tetracaine								
	0.25%	5.2	4.0	3/5	2/5	--	206(2) 41/2<24 hrs. (1)	180(2)
T/Tet	1/0.25	5.9	5.5	4/5	1/5	--	4<24 hrs.	
T/Tet	3/0.25	6.4	5.0	5/5	--	--	3<24 hrs.	

See Notes under Table I.
B = Bupivacaine; Pr = Prilocaine; Tet = Tetracaine.

EXAMPLE 2

The use of anesthetics of the present invention is also shown through peridural blocks in the cat. The surgical techniques and testing methods have been described in detail (Duce et al.: Brit. J. Anaesth., Vol. 41, 579-587 (1969)). The animals were treated according to the following scheme in this study:

Cat No.	Weight and Sex	Day (treatment) 1	2	3	4	5
124	3.6 kg F	X	L	TTX	L/TTX	X
125	2.8 kg F	X	L	L/TTX	TTX	X
127	4.0 kg M	X	TTX	L	L/TTX	X
128	2.8 kg F	X	TTX	L/TTX	L	X

X = Xylocaine HCl, 2% as salt
L = Lidocaine HCl, 2% as base
TTX = Tetrodotoxin, 1 .mu.g/ml
F = female
M = male

All animals were tested with 2 percent Xylocaine (a commercial local anesthetic composition based on lidocaine as the active ingredient) on Days 1 and 5 to ascertain the stability of the peridural cat preparation. Within the test period, laboratory-prepared samples of lidocaine were used containing only lidocaine and epinephrine or lidocaine, epinephrine and tetrodotoxin in specified proportions. Solutions were freshly prepared each day of use; epinephrine was added and the pH taken shortly before administration. The pH's of the solutions were: tetrodotoxin, 4.5-6.75; lidocaine HCl, 4.75-4.8; lidocaine/tetrodotoxin, 4.75-4.9.

The results are summarized in Table IV. In general, no overt systemic effects were noted following administration of the test solutions. Animal No. 127 exhibited salivation and emesis, with bile present, about 3 hours and 45 minutes after administration of the lidocaine/tetrodotoxin combination. However, these observations were not considered significant.

Statistical analysis of the data showed that the Xylocaine control values obtained on days 1 and 5 are not significantly different. Since tetrodotoxin alone produced no blocks, it was excluded from the analysis of variance in order to keep the variance reasonably homogeneous. A four-way analysis of variance was, therefore, done only with the data obtained with 2 percent lidocaine and with the lidocaine/tetrodotoxin combination. The durations of block with the lidocaine tetrodotoxin combination were statistically significantly longer than with lidocaine itself.

TABLE IV

PERIDURAL ANESTHESIA IN CAT

Compound and Concentration	Deep Motor Block Duration X .+-. S.E.	Onset	Frequency	Block of Support of Weight Duration X .+-. S.E.	Onset	Frequency	Block of Flexion Reflex Duration X .+-. S.E.	Onset

									Frequency
2% Xylocaine (Day 1)	119 .+-. 12	1	8/8	98 .+-. 13	<1	8/8	48 .+-. 10	8	4/8
2% Xylocaine (Day 5)	124 .+-. 11	1-2	8/8	106 .+-. 9	1	8/8	70 .+-. 17	7	6/8
1 .mu.g/ml Tetrodotoxin	--	--	0/8	--	--	0/8	--	--	0/8
2% Lidocaine	115 .+-. 10	2-3	8/8	88 .+-. 10	2	8/8	66 .+-. 19	7	5/8
Lidocaine/tetrodotoxin	226 .+-. 9	<1	8/8	188 .+-. 9	<1	8/8	109 .+-. 8	7	7/8

Durations are in minutes.
Mean onset times are approximate.
Durations of block of flexion reflex in this table were calculated withou zero values.
All solutions contained 1:100,000 epinephrine.

EXAMPLE 3

The effectiveness of the local anesthetics of the present invention in the absence of epinephrine is shown by the data set forth in Tables V, VI and VII. The data summarized in these tables were obtained following the same procedures as described in Example 1.

Five separate studies were done, and a tetrodotoxin control group was run in each study. Frequency of block with tetrodotoxin ranged from 0/5 to 3/5, and durations ranged from about 180 to 240 minutes. Frequency of block was 5/5 with all combinations except that containing phenacaine (Table VI). The one partial block in this case may have been due to failure to inject the solution sufficiently close to the sciatic nerve trunk. The frequency of block with the diperodon-tetrodotoxin, cyclomethycaine-tetrodotoxin and dibucaine-tetrodotoxin were better than with diperodon, cyclomethycaine, dibucaine or tetrodotoxin by itself.

In all cases, the combinations produced durations markedly longer than obtained with the local anesthetics alone. The durations of tetrodotoxin alone were closer to those with the combinations; the frequencies were consistently lower than those produced by the combinations.

TABLE V

RAT SCIATIC NERVE BLOCKS

Compound	% Conc. as base	pH	Onset (min.)	Frequency C	Frequency P	Duration (min.) Mean .+-. S.D. C.R.	Duration (min.) Mean .+-. S.D. R.G.
Lidocaine	2.0	4.8	1	5/5	--	108 .+-. 48	103 .+-. 37

Lidocaine/TTX	2.0	4.8	<1	5/5	--	385 .+-. 25	348 .+-. 17
Procaine	2.0	5.5	2.5	5/5	--	62 .+-. 7	60 .+-. 7
Procaine/TTX	2.0	5.5	2	5/5	--	356 .+-. 53	314 .+-. 58
Chloroprocaine	2.0	5.4	1.5	5/5	--	111 .+-. 65	87 .+-. 36
Chloroprocaine/TTX	2.0	5.3	1	5/5	--	351 .+-. 73	325 .+-. 46
Tetrodotoxin	2 .mu.g/ml	6.0	13	2/5	0/5	242 .+-. 4	226 .+-. 5
Diperodon	0.25	5.4	22	2/5	0/5	124	64
Diperodon/TTX	0.25	5.4	9	5/5	--	332 .+-. 59	286 .+-. 33
Propoxycaine	0.25	5.4	4	5/5	--	64 .+-. 12	52 .+-. 15
Propoxycaine/TTX	0.25	5.5	1.5	5/5	--	262 .+-. 91	239 .+-. 102
Hexylcaine	0.5	5.6	3	5/5	--	112 .+-. 12	99 .+-. 17
Hexylcaine/TTX	0.5	5.5	4.5	5/5	--	339 .+-. 17	303 .+-. 12
Cocaine	0.25	6.1	4.5	5/5	--	98 .+-. 9	86 .+-. 17
Cocaine/TTX	0.25	5.6	5	5/5	--	(1 day)	361 .+-. 28
Tetrodotoxin	2 .mu.g/ml	6.1	16	2/5	1/5	216 .+-. 51	161

TTX = Tetrodotoxin, 2 .mu.g/ml; C = Complete block; P = Partial block; C.R. = Complete recovery of normal motor function; R.G. = Recovery of grasping; Durations are for complete blocks only; Onset times are approximate.

TABLE VI

RAT SCIATIC NERVE BLOCKS

Compound	% Conc. as base	pH	Onset (min.)	Frequency C.	Frequency P	Duration (min.) Mean .+-. S.D. C.R.	Duration (min.) Mean .+-. S.D. R.G.
Phenacaine	0.25	5.6	4.5	5/5	--	78 .+-. 32	70 .+-. 32
Phenacaine/TTX	0.25	5.5	8	4/5	1/5	282 .+-. 74	253 .+-. 71
Benoxinate	0.25	5.6	3	5/5	--	116 .+-. 24	

							97 .+-. 20
Benoxinate/TTX	0.25	5.6	8	5/5	--	320 .+-. 54	285 .+-. 58
Butacaine	0.25	5.8	6	4/5	1/5	73 .+-. 7	67 .+-. 2
Butacaine/TTX	0.25	5.6	5	5/5	--	241 .+-. 24	204 .+-. 37
Tetrodotoxin	2 .mu.g/ml	6.1	18	1/5	--	181	150
Proparacaine	0.5	6.0	1.5	5/5	--	98 .+-. 20	89 .+-. 14*
Proparacaine/TTX	0.5	6.1	2	5/5	--	429 .+-. 41	415 .+-. 50*
Tetrodotoxin	2 .mu.g/ml	6.2	13	3/5	2/5	222 .+-. 48	206 .+-. 42

TTX = Tetrodotoxin, 2 .mu.g/ml; C = Complete block; P = Partial block; C.R. = Complete recovery of normal motor function; R.G. = Recovery of grasping; Durations are for complete blocks only; Onset times are approximate.
*Means of 3 animals; 2/5 died.

TABLE VII

RAT SCIATIC NERVE BLOCKS

Compound	% Conc. as base	pH	Onset (min.)	Frequency C	Frequency P	Duration (min.) Mean .+-. S.D. C.R.	Duration (min.) Mean .+-. S.D. R.G.
Cyclomethycaine	0.125	5.1	17	1/5	4/5	145	115
Cyclomethycaine/TTX	0.125	5.2	5	5/5	--	273 .+-. 43	231 .+-. 41
Dibucaine	0.125	5.4	6	3/5	1/5	125 .+-. 22	108 .+-. 25
Dibucaine/TTX	0.125	5.4	6	5/5	--	324 .+-. 47	272 .+-. 56
Tetrodotoxin	2 .mu.g/ml	5.6	--	0.5	1/5	--	--

TTX = Tetrodotoxin, 2 .mu.g/ml; C = Complete block; P = Partial block; C.R. = Complete recovery of normal motor function; R.G. = Recovery of grasping; Durations are for complete blocks only; Onset times are approximate.

EXAMPLE 4

The use of desoxytetrodotoxin was tested following the procedure described in Example 1. The desoxy derivative was substituted for the tetrodotoxin referred to in Example 1. Desoxytetrodotoxin was tested, without epinephrine, in rat sciatic nerve blocks. At concentrations of 5, 10 and 20 .mu.g/ml it produced no blocks. The duration of block of a combination containing 2 percent lidocaine and 5 .mu.g/ml of desoxytetrodotoxin was not significantly different from that of 2 percent lidocaine alone. However, combinations containing 10 and 20 .mu.g/ml of desoxytetrodotoxin produced blocks that were significantly longer (1.4-1.6 times) than that of lidocaine alone (0.008> p> 0.016).

This result is to be expected based on the tests of tetrodotoxin in view of the lower activity shown by the desoxy derivative in toxicity tests. Literature on the toxicity of tetrodotoxin and its desoxy derivative reports the latter to be between one quarter and one tenth as toxic as its parent toxin.

EXAMPLE 5

Method: Mature male beagles are surgically prepared by implantation of a cannula into a lumbar vertebra so that drug solutions may be administered into the peridural space. After administration of local anesthetic solutions, the animals are examined at intervals for duration of loss of pain in the scrotal area and in the digits of the hind limbs as well as for loss of ability to suport their weight.

Response to and awareness of pain stimuli in scrotal area is a test for anesthetic block in spinal roots L3-4 and S1-2-3. These roots are the furthest removed from the point of injection (L6) and, therefore, least likely to be affected by the anesthetic. Return of response to pain in the scrotum is often the first sign of recovery and indicates recession of anesthesia to at least L4 anteriorly and S2 posteriorly.

TABLE VIII

PERIDURAL ANESTHESIA IN DOGS

Compound and Concentration	Onset: mean and range Digital Pain	Scrotal Pain	Weight Support	Duration: mean and range Digital Pain	Scrotal Pain	Weight Support
Lidocaine 2% (n = 3)	7	8	<5	127 76-162	111 62-152	137 108-162
Tetrodotoxin 4 .mu.g/ml (n = 2)	19.5 19-20	15*	<17	225 87-350	0-125	406 339-473
Lidocaine 2% Tetrodotoxin	<5	<5	<3	316 245-387	301 235-367	462 400-525

*One animal only; no anesthesia in second animal.
Onsets and durations are in minutes. All Solutions contained 1:100,000 epinephrine. Volume of administration = 5 ml. n = number of animals
NOTE:

(1) With lidocaine onset is rapid, frequency of block is 100%, but durations are short.
(2) With tetrodotoxin durations are long, but onset is slow and frequency of block of scrotal pain is poor.
(3) With the combination onset is rapid, frequency is 100% and durations are long.

EXAMPLE 6

Following the method described in Example 1 above, various local anesthetic compounds alone, TTX alone and combinations of the compounds with TTX were tested for their ability to block the rat sciatic nerve. TTX was used uniformly in the amount of 2 .mu.g/ml. Each of the compositions tested contained epinephrine in concentration of 1:100,000. The results are presented in Table IX. In the case of compound A in 0.5% concentration, duration was about 126 minutes. TTX alone was about 295 minutes but frequency was not good. In combination, frequency was good and duration was greater than 420 minutes.

In the case of compound D at 0.25% concentration, duration was about 128 minutes alone but greater than 420 minutes in combination with TTX. In the case of compound E at 0.25% concentration, no blocks were observed alone, but in combination with TTX the duration was about 148 minutes. In the case of compound F alone at 0.125% concentration, duration was only 78 minutes with poor frequency, whereas in combination with TTX duration was greater than 322 minutes and frequency had improved. For compound G at 0.5% concentration, duration was 104 minutes alone and about 286 minutes in combination with TTX.

It should be noted moreover that in the case of TTX alone, the frequency and duration were quite variable ranging from zero frequency to 4 out of 5, and ranging from zero duration to 295 minutes or more.

Table IX

Rat Sciatic Nerve Blocks

Tetrodotoxin (TTX) (2 .mu.g/ml) and Various Local Anaesthetic Compounds. Epinephrine concentration 1:100,000.

Compound	Frequency	Duration (min.) Mean .+-. S.D.
TTX	2/5	295*
A (0.5%)	5/5	126 .+-. 12
TTX + A (0.5%)	5/5	>420, <24 hrs.**
A (1.0%)	5/5	157 .+-. 18
TTX + A (1.0%)	5/5	>420, <24 hrs.
TTX	4/5	316 .+-. 10*
D (0.25%)	5/5	128 .+-. 13
TTX + D (0.25%)	5/5	>420, <24 hrs.
D (0.5%)	5/5	133 .+-. 7
TTX + D (0.5%)	5/5	>420, <24 hrs.
TTX	0/6	0
E (0.25%)	0/6	0
TTX + E (0.25%)	4/6	148 .+-. 27
TTX	0/5	0
F (0.125%)	1/5	78
TTX + F (0.125%)	3/5	>322 min.
TTX	0/5	0

G (0.5%)	5/5	104 .+-. 14
TTX + G (0.5%)	4/5	286 .+-. 197

*One animal blocked >420 min.
**>420, <24 hrs. means that the animals returned to normal during a perio when they were not observed, this period being longer than 7 hrs. and shorter than 24 hrs.

EXAMPLE 7

In vitro tests were made on the isolated intact frog sciatic nerve using compounds B, C and lidocaine alone and in combination with TTX. The results and the method followed are presented in Table X. The reduction in the action potential of compound B alone was 22% and for TTX alone it was 15%, as compared with a reduction of 94% for the combination. For compound C alone the reduction was 24%, and for TTX alone 29%, whereas the combination again reduced the potential by 94%. For lidocaine and TTX each alone the reductions were 15% and 7%, respectively, as compared with a reduction of 61% for the combination of the two.

Table X

Block of Isolated Intact Frog Sciatic Nerve.

Compound	pH	Concn. mM	Percent reduction of the action potential. Mean and range	Number of experiments
B	5.6	0.625	22 (10-38)	16
TTX	5.6	3 .sup.. 10.sup..sup.-4	15 (8-)	17
B + TTX	5.6	as above	94 (80-100)	17
C	5.6	0.156	24 (15-52)	8
TTX	5.6	3 .sup.. 10.sup..sup.-4	29 (14-80*)	6
C + TTX	5.6	as above	94 (78-100)	12
Lidocaine	7.0	0.625	15 (6-30)	6
TTX	7.0	1 .sup.. 10.sup..sup.-4	7 (2-12)	6
Lidocaine + TTX	7.0	as above	61 (20-100)	12

*Occasionally a high value is observed, probably caused by a minute damag to the nerve sheath during dissection. It takes about 50 times the concentration of TTX which is necessary to block a desheathed nerve in order to obtain the same degree of block of an intact (sheathed) nerve.

Method: The method is essentially as described by A. P. Truant, Arch. Int. Pharmacodyn. 115, 483-497 (1958).

Sciatic nerve trunks of Rana pipiens are prepared by dissecting the nerve from its roots in the spinal cord to the ankle and placing it on silver-silver chloride electrodes so that stimulation and recording of the action potential can be performed during the course of application of the test compounds and during the recovery period. The bathing solution is Tasaki Ringer's. The observations lasted for 40 minutes

allowing the action potentials to reach essentially a stable value (equilibrium).

EXAMPLE 8

Using the procedure described in Example 1 above, the effect of several known vasoconstrictors on rat sciatic nerve blocks was investigated using lidocaine (0.125%) and tetrodotoxin (2 .mu.g/ml) in combination. The results are given in Table XI. Without any vasoconstrictors, the frequency was very poor and the duration of block was 174 minutes. With phenylephrine, levonordefrin, or epinephrine, however, frequency was greatly improved and duration had about doubled.

Table XI

Effect of Vasoconstrictors on Rat Sciatic Nerve Blocks Obtained with Lidocaine (0.125%) and Tetrodotoxin (2 .mu.g/ml).

Vasoconstrictor	Concn.	Frequency	Duration of Block (min.) Mean .+-. S.D.
None	--	1/5	174
Phenylephrine	1:20,000	5/5	377 .+-. 27
Levonordefrin	1:20,000	5/5	354 .+-. 12
Epinephrine	1:200,000	5/5	368 .+-. 24

EXAMPLE 8a

Using the procedure described in Example 1, except that no epinephrine was added to the solutions tested, the local anesthetics falicain and pramoxine were tested for blockage on the rat sciatic nerve alone and in combination with TTX at 2 .mu.g/ml. The results are presented in the following Table XII.

TABLE XII

Rat Sciatic Nerve Blocks

	Frequency	Duration
0.25% falicain	5/5	55 .+-. 22
0.25% falicain .+-. TTX, 2 .mu.g/ml	5/5	116 .+-. 71
0.25% pramoxine	0/5	0
0.25% pramoxine .+-. TTX, 2 .mu.g/ml	2/5	190 .+-. 76
TTX, 2 .mu.g/ml	0.5	0

It will be observed that the ingredients were tested at dose level that did not result in any anesthesia at all for two of them, and only 55 min. for the third one, whereas the combination gave anesthesia about 2 to 3 hrs. The frequency of complete block was raised from 0 to 40% in the case of pramoxine.

Compounds A, B, C, D and L described in Table A above are made by the procedure described in U.S. patent application Ser. No. 369,146, filed June 12, 1973, which is a continuation-in-part of Ser. No. 325,378, filed Jan. 22, 1973, now abandoned, both assigned to the same assignee as the present

application, which disclosure is incorporated herein by reference.

The method of preparing compounds S and T is disclosed in U.S. patent application Ser. No. 164,022 filed July 19, 1971, now U.S. Pat. No. 3,812,147, which is incorporated herein by reference.

The method of preparing compound Q is disclosed in U.S. patent application Ser. No. 321,590 filed Jan. 8, 1973, now abandoned, which is incorporated herein by reference.

Compounds H, J and M and mepivacaine are known compounds disclosed in the published literature.

EXAMPLE 9

Synthesis of 2-(N-ethyl-isopropylamino)-2', 6'-propionoxylidide (Compound E)

A mixture of 12.81 g (0.050 mole) of 2-bromo-2', 6'-propionoxylidide, 11.31 g (0.130 mole) ethyl-isopropylamine and 30 ml dry toluene was heated in a glass-lined, stainless-steel pressure vessel at 105.degree. for 20 hours. After cooling to 25.degree., the brown reaction mixture was filtered, extracted three times with a total of 50 ml of 3 N HCl. The aqueous solution was heated to 75.degree. for 10 minutes with decolorizing carbon and then filtered. To the chilled solution was added 10 ml concentrated NH.sub.3. The product which precipitated was filtered, washed, and dried. Yield: 6.93 g (52.9%) m.p. 50-2.degree..

Anhydrous ethereal HCl was added to 6.90 g of the above base dissolved in 100 ml dry ether until the solution was acidic to moist pH paper, giving 6.15 g of tacky brown material, m.p. 191.degree.-201.degree.. The hydrochloride was recrystallized from a mixture of butanone and 2',6'Yield: 6.02 g, m.p. 207.5.degree.-209.degree..

Analysis: Calc'd for C.sub.16 H.sub.27 ClN.sub.2 O: C 64.30, H 9.11, N 9.37, Cl 11.86. Found: C 64.16; H 9.16, N 9.49, Cl 12.09.

EXAMPLE 10

A. Synthesis of 2-Bromo-4'-butoxy-2', 6'-dimethyl propionanilide

To a chilled (ca 10.degree.) solution of 50.7 g (0.263 mole) of 4-butoxy-2,6-dimethylaniline [Buchi et al., Helv. Chim. Acta, 34, 278 (1951)] in 224 ml glacial acetic acid was added rapidly 62.4 g (.289 mole) of 2-bromo-propionyl bromide and immediately thereafter a chilled (ca 5.degree.) solution of 87.2 g sodium acetate trihydrate in 362 ml water. This mixture was shaken for 1/2 hour, filtered, washed with water until the washes were neutral, and dried in vacuo over silica gel and KOH; yield 68.9 g (71.6%); m.p. 132.5.degree.-133.5.degree.. The product was recrystallized from 95% ethanol; m.p. 135.5.degree.-136.degree..

Analysis: Calc'd for C.sub.15 H.sub.22 NO.sub.2 Br: C 54.87, H 6.76, Br 24.34. . Found: C 55.06, H 6.22, Br 24.69.

B. Synthesis of 2-Methylamino-4'-butoxy-2',6'-dimethyl-propionanilide (Compound F)

To a cold stirred solution of 14.8 g. of monomethyl amine in 250 ml dry benzene was added (portionwise, keeping temperature below 10.degree.) 19.5 g (0.0594 mole) of 2-bromo-4'-butoxy-2', 6'-dimethyl propionanilide (made according to the procedure in the first part of this example); this dissolved readily forming a clear solution. The mixture was heated to 70.degree. for ca 1 hr. with stirring, at which point a white precipitate had separated and reflux became so vigorous that the reaction had to be controlled by external cooling.

The precipitated methylammonium bromide was filtered off. Excess amine and solvent were removed in vacuo from the filtrate, giving a white residue which was dissolved in 120 ml 0.5 M HCl and filtered. The filtrate was extracted with 3.times. 25 ml. ether; and the ether extracts discarded.

The aqueous phase was alkalized to pH 11, and extracted with ether; the combined extracts were dried (Na.sub.2 SO.sub.4), filtered, and evaporated, giving a yield of 8.7 g (52.7%); m.p. 107.degree.-107.5.degree.. Recrystallization from cyclohexane did not effect the melting point.

Analysis: Calc'd. for C.sub.16 H.sub.26 N.sub.2 O.sub.2 : C 69.0; H 9.41 ; N 10.06. Found: C 69.0; H 9.17; N 10.06.

EXAMPLE 11

Synthesis of 2-(N-Methyl-n-propylamino)-2', 6'-butyroxylidide (Compound G)

To a stirred solution of N-methyl-n-propylamine (9.10 g, 0.125 mole) in 175 ml of anhydrous benzene was added 2-iodo-butyro-2',6'-xylidide (13.2 g, 0.0415 mole). The mixture was allowed to reflux for 5 hrs.

The reaction mixture was extracted with 1 M HCl. After filtration to remove trace insolubles, the pH was adjusted to 9 with 7 M NaOH, which caused the formation of a light-yellow waxy solid. The latter was filtered, washed with water, and dried; yield 4.00 g (36.7%).

This base was converted to the hydrochloride salt with ethereal HCl. The hydrochloride was twice-recrystallized from ethanol/ether, affording cyrstals melting at 214.degree.-215.degree. C.

Analysis: Calc'd. for C.sub.16 H.sub.27 ClN.sub.2 0: C 64.3; H 9.11; Cl 11.86 Found: C 64.4; H 9.01; Cl 11.80.

EXAMPLE 12

Synthesis of 2-Cyclobutylamino-2', 6'-acetoxylidide (Compound K).

To a solution of cyclobutylamino (39.8 g) in 600 ml benzene was added 2-chloro-2'-acetoxylidide (49.4 g), slowly, with stirring, and the mixture was refluxed for about 5 hrs. After cooling, the mixture was filtered to remove the cyclobutylammonium chloride formed. The filtrate was stripped of solvent and excess amine in vacuo; leaving a crude residue.

The residue was dissolved in 0.5 M hydrochloric acid, the solution was made alkaline with sodium hydroxide solution and the base was extracted carefully with ether. The ether solution was dried (Na.sub.2 SO.sub.4), the ether and low-boiling components were evaporated in vacuo at 40.degree.-50.degree. C. and the residue converted to a hydrochloride by addition of ethereal hydrogen chloride to its filtered ether solution. From the hydrochloride the base was obtained by dissolution in water, addition of sodium hydroxide solution to alkaline pH, extraction with ether, drying of the ether extract (Na.sub.2 SO.sub.4), filtering, and evaporation of the ether. The base could be recrystallized from cyclohexane, petroleum ether (b.p. 60.degree.-110.degree. C.), or heptane. The melting point was found to be 75.degree.-78.degree. C.

Analysis: Calc'd. for C.sub.14 H.sub.20 N.sub.2 O : C 72.4, H 8.68, N 12.06. Found: C 72.4, H 8.88, N 11.93.

EXAMPLE 13

A. Synthesis of 2-(sec-butylamino)-2', 6'-acetoxylidide

To a solution of 62.2 g of sec-butylamine in 500 ml benzene was added slowly 41.5 g of 2-chloro-2', 6'-acetoxylidide. The mixture was heated to reflux for 7 hours and allowed to cool overnight. The precipitate of sec-butyl amine hydrochloride that formed was filtered off and the filtrate was evaporated to an oily residue. The residue was dissolved in ether, and the solution was filtered, dried (Na.sub.2 SO.sub.4), and evaporated to an oily residue (45.7 g). This crude product was distilled under vacuum,

giving an oily liquid that solidified when chilled. Yield: 38.5 g (78%); b.p. 146.degree./0.05 mm; m.p. 44.5.degree.-45.5.degree..

Analysis: Calc'd. for C.sub.14 H.sub.22 N.sub.2 O : C 71.75, H 9.46, N 11.96. Found: C 71.99, H 9.35, N 12.12. The hydrochloride melted at 176.5.degree.-178.5.degree.. ##STR28##

B. Synthesis of 2-(N-ethyl-sec-butylamino)-2', 6'-acetoxylidide (Compound P)

To 140 g of diethyl sulfate was added 30.5 g of 2-(sec-butylamino)-2', 6'-acetoxylidide (made by the method described in the first part of this example). The mixture was heated to 100.degree.-110.degree. for 5 hours and cooled. Water and 5 N HCl were added to pH 2, forming a second phase. After stirring, the aqueous phase (pH 2) was separated, washed with two 100 ml portions of ether and brought up to pH 9 with concentrated NH.sub.3. The basic aqueous phase was extracted with five 100 ml portions of ether. The solvent was stripped in vacuo from the combined ether phases, leaving a solidifying oil which was dissolved in ether, dried (Na.sub.2 SO.sub.4), filtered, and evaporated in vacuo. Yield: 26.2 g (76.8%); m.p. 50.5.degree.-54.5.degree. . The product was twice distilled under high vacuum : b.p. 147.degree. /0.025 mm; 165.degree./0.4 mm. Yield of redistilled product: 21.4 g (62.7%).

Analysis: Calc'd. for C.sub.16 H.sub.26 N.sub.2 O : C 73.23%, H 9.99%, N 10.68%. Found: C 73.06%, H 9.66%, N 10.47%.

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